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SUB-THEME III

Diversify Utilization of Natural Resources for Sustainable Development

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FOREWORD

We would like to thank His Excellency Dr. Myo Thein Gyi, Hon'ble Union Minister for Education, for delivering the inaugural address at the Opening Ceremony of the 19th Science Council of Asia Conference, and for the support His Excellency had given for holding this international conference, for the first time, in Myanmar. We are also indebted to express our heartfelt gratitude to His Excellency Dr Wang Xi, Vice Minister, Ministry of Science and Technology of China, President, Science Council of Asia, and Vice President, China Association for Science and Technology (CAST) for his presence at this opening ceremony. We would also like to extend our gratitude to Professor Dr Hiroshi YOSHINO, the Secretary General/Treasurer of SCA. Special thanks would go to Dr Juichi Yamagiwa, the President of SCJ and to all distinguished guests and scholars from SCA member academies who, in one way or the other, contributed to the success of this conference.

MAAS owes its apology to all participants for the delay in producing the papers presented at SCA–19 Conference. The responsibility of opinions, statements, comments, etc. expressed in the papers lie with those of the authors. The views expressed in the papers presented at the conference did not reflect that of MAAS.

Dr Thet Lwin

President, Myanmar Academy of Arts and Science

Lists of Contributors

Message

It is a great pleasure and inspiring experience to host the 19th Science Council of Asia Conference, held with the theme "Research and Innovation for Sustainable Development in Asia" in Nay Pyi Taw, Myanmar.

This conference is particularly timely as all Member States are unanimously supporting research and innovation to achieve the Sustainable Development Goals (SDGs) of the United Nations 2030 Agenda.

A comprehensive range of the conference sessions reflects a very topical agenda as it provides a wonderful platform and opportunity for several experts, academia, scholars and researchers to exchange their break–through ideas, profound knowledge and invaluable experiences.

I sincerely hope this conference will help to identify ways to bring forward the transformative sustainability agenda and better align research and innovation policies with the SDGs, as set out in the United Nations Agenda 2030 for sustainable development.

To all delegates, I would like to suggest that we adopt innovation in science, technology, engineering and mathematics as integral elements of the strategies for SDGs. I would like to request you to produce action–oriented outcomes of the conference and keep supporting research and innovation for sustainable development in Asia.

Before I conclude, I wish all delegates and participants an enjoyable stay in Myanmar.

Thank you.

Dr Myo Thein Gyi
Union Minister for Education, Myanmar

Message

It is my great pleasure to welcome you all on behalf of Science Council of Asia(SCA) to the 19th SCA conference that brings together experts of scientific organizations to discuss " Research and Innovation for Sustainable Development in Asia". I thank all the local conference hosts in Myanmar and the SCA Secretariat. Thanks to their excellent effort, the SCA19 is organized jointly and successfully.

To a large extent, Asia's rapid economic development and subsequently booming consumption have prompted critical challenge for environment sustainability. Recently, Oxford Dictionaries and Cambridge Dictionary have named "climate emergency" and "upcycling" as their Word of the Year 2019, respectively. Change is necessary.

The 2030 Agenda, adopted at the United Nations Sustainable Development Summit in September 2015, positioned Science, Technology and Innovation as key means of implementation of the SDGs. Also with a new perspective of economic growth, there is a clear shift towards appreciating the adoption of sustainability standards, which has a positive correlation with increased profit as demand for products with sustainable credentials grows.

Many Asian countries have signed up to the SDGs, made growing commitment to address poverty, clean energy and pollution. New science and technology achievements have been applied to solve specific issues, new approaches have been used towards more sustainable activities.

SCA provides such a collaborative platform for member organizations to promote scientific exchange and cooperation, and to reach mutual understanding. We will continue to play a central role to face the common challenges for sustainable development in Asia and lead to a bright future.

Wishing you all a fruitful and joyful stay in Nay Pyi Taw and looking forward to your active participation in the Conference.

With best regards,

Prof. Dr. WANG Xi
President, Science Council of Asia

Message

Millennium Development Goals (MDGs) came to an end in December 2015; certain agenda of MDGs had been left unfinished. In 2015, UN General Assembly adopted the post 2015 Development Agenda and set Sustainable Development Goals (SDGs). The 2030 Global Development Agenda came into effect on 1st January, 2016 with the prospect of covering the five aspects: People, Planet, Prosperity, Peace and Prosperity. The approach to SDGs can be initiated through a different angle. For instance, People: End Poverty and Hunger in all forms and ensure dignity and equality; Planet: Protect our planet's natural resources and climate for future generations; Peace: Foster peaceful, just and inclusive societies; Prosperity: Ensure prosperous and fulfilling lives in harmony with nature.

Living beings inherited the earth, the only planet in the solar system, in which all known life forms can flourish. Human beings are the most intelligent species on earth. Throughout history, they try to change their environment to suit their needs and satisfy their desires. Among the continents, Asia happens to be the most populous region in the world. China and India, each with one billion plus population, have emerged as economic powers. Japan, Korea, Taiwan, and ASEAN TEN too witnessed economic growth. Inventions and innovations contributed to industrial revolutions. The fourth forthcoming inventions of IT and AI are expected to change traditional modes of manufacturing, transportation and urban amenities.

In 21st century, research across various countries in Asia highlight inter–linkages between natural resources and sustainable development goals along with the impact of climate change. The human society is seeking ways to safeguard human's wellbeing and to guarantee the sustainable use of resources. At the same time, it also tries to conserve the environment from natural calamities like floods, earthquakes, volcanic eruptions, drought, etc. so that our future generations will be inherited with green and peaceful environment.

I, on behalf of Myanmar Academy of Arts and Science, would like to express my deep appreciation to all those involved in making the conference a success. Special mention needs to be made to SCJ which co–hosted the SCA19 conference with MAAS for their guidance, support, co–operation and coordination. We fervently believe that the conference will provide our guests a cordial atmosphere for sharing experiences and exchanging views. The deliberations from this conference are expected to bring into light, effective means and ways for resolving common issues people are currently facing in Asia and elsewhere.

Dr. Thet Lwin
President of MAAS

Message

I am pleased to announce that the 19th Science Council of Asia (SCA) Conference is held in Nay Pyi Taw, Myanmar. First of all, I would like to express my sincere gratitude to Myanmar Academy of Arts and Science (MAAS) for its enormous efforts to prepare for this Conference. I also would like to express my deep appreciation to Member Organizations and SCA Secretariat for their cooperation and contribution in organizing this Conference and all the people who support it.

The theme of the 19th SCA Conference is "Research and Innovation for Sustainable Development in Asia". As you might already know, the purpose of SCA established in 2000 is "to facilitate scientific cooperation in Asia towards the progress in science and sustainable development of the region". With this purpose, SCA has contributed to society through the collaboration with academies in the region. Under such circumstances, "Transforming our world: the 2030 Agenda for Sustainable Development" with the 17 Sustainable Development Goals (SDGs) and 169 targets was adopted at the UN General Assembly in 2015. Since then, the efforts to realize the sustainable development in which a key principle is "no one will be left behind" have started at global level. We must continuously and collaboratively address a wide range of issues facing us since they are so challenging in the sense that human beings have never experienced them before.

The 19th SCA Conference is held for 3 days and nearly 180 presentations (oral and poster) are scheduled. I am delighted to know that we have received many applications exceeding our expectation. It indicates a high level of interest in this Conference's theme, "Research and Innovation for Sustainable Development in Asia". In the Conference, there are four (4) sub–themes that are discussed in their respective sessions. The main theme and sub–themes of the Conference have been carefully selected in order to inspire scholars and researchers in Asia to undertake interdisciplinary researches in partnership to contribute to the realization of the 17 SDGs.

This Conference is an advantageous opportunity to build a network of scholars in and outside the country. I am sure that there would be abundant outstanding discussions at this Conference.

Professor Hiroshi Yoshino

Secretary General / Treasure of SCA

Distribution and Status of Sarus Crane G*rus Antigone* in wetlands of Ayeyarwady Region

Myo Sandar Win[1] et al

Abstract

The present research conducts with the distribution pattern and status of globally threatened vulnerable species of Sarus Crane *Grus antigone* in Ayeyarwady Region. Data was recorded by direct observation and interview survey method. Sarus Cranes have been distributed in 14 Townships (135 villages) in breeding season (June–October) and non-breeding season (November-May) in four districts. According to the result, 395 individuals of Sarus Crane and 127 nests were recorded in during the period of June 2015 to May 2018. Out of them, 245 individuals of adult and 54 individuals of juvenile were recorded in non – breeding season. Nests were recorded in 57 villages. During the study period, highest population number of Sarus Crane and their nests were recorded in Wakhema and Maubin Township. Habitats were also recorded. Evaluating the breeding status of Sarus Cranes by Mayfield (1979) method, hatching (80.1%), crude breeding success (55.7%) and fledging success (69.6%) were calculated.

Keywords: Ayeyarwady, breeding, habitats, Sarus Crane, wetland

1. Introduction (12P't, Times New Roman)

Ayeyarwady Region has an area of 35,140 square kilometres (13,566 sq. miles). It is the very vast region, there are 26 Townships comprising with 1913 villages under the six Districts. Sarus Crane is currently listed as vulnerable species on the IUCN Red list. It is conspicuous and iconic species of open wetlands. Wetland – dependent birds need functional assess to a wetland or wetland products almost totally for breeding, nesting, feeding, or shelter during their breeding cycles. Wetlands are still being degraded in many parts of the world (Schuyt, 2005). The purpose of the research is to conduct the Sarus Crane distribution and status on population and habitats.

2. Methods

Study area

Ayeyarwady region lies between north latitude 15° 40' and 18° 30' approximately and between east longitude 94° 15' and 96° 15'. Data were recorded in four districts (Maubin, Myaungmya, Pyapon, and Latbutta).

Study period

The survey was conducted from June, 2015 to May, 2018.

Field data collection

Population surveys conducted towards the end of the breeding season. At each Crane

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site, habitats were collected by rapid assessment by level 4 of Asian Wetland Inventory Handbook (Finlagson *et al.*, 2002). Data were collected using by direct observation and interview survey method. Interviewed surveys conducted with a wide variety of people such farmers, villagers, boatmen, fishermen and other local people who well known about the Crane.

Data analysis for breeding status

Breeding status was analyzed by method of Mayfield, 1979.

$$\text{Hatching success (\%)} = \frac{\text{Number of chicks}}{\text{Total number of eggs}} \times 100$$

$$\text{Fledging success (\%)} = \frac{\text{Number of fledging birds}}{\text{number of chicks}} \times 100$$

$$\text{Crude breeding success (\%)} = \frac{\text{Number of fledging}}{\text{Total number of eggs}} \times 100$$

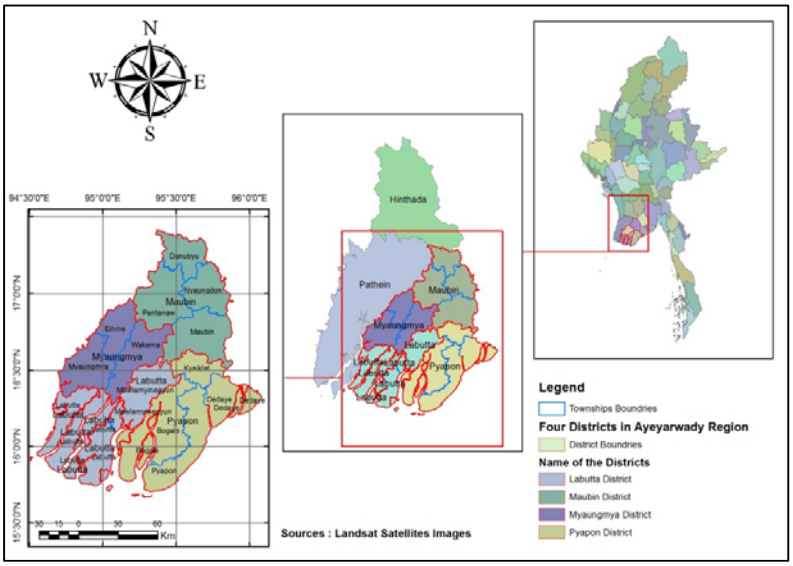

Figure 1. Map of the study sites

3. Results and Discussion

Sarus Crane' distribution and their status

The research was conducted in four districts of Ayeyarwady Region (135 villages in 14 townships). Sarus Cranes have been distributed in four districts in breeding season (June–October) and non-breeding season (November-May). Highest population Crane numbers and nests were recorded in Wakhema Township and followed by Maubin Township (Figure 1). The habitats of both townships were suitable habitats for Sarus Crane. According to the survey result, 395 individuals (245 adult, 54 juvenile) of Sarus Crane and 127 nests were recorded in 14 Townships during the research period (Figure 2 and Table 1). Lowest population number and nest were recorded in other Townships. In these Townships, habitats were not sufficient for roosting, foraging and nesting.

Breeding status of Sarus Crane

During the research period, nests were recorded in 57 villages. Nesting sites were mostly found in natural wetlands and flooded paddy field. The natural wetlands appear to be the most preferred habitats for nesting and were crucial to ensure maximum breeding success of Sarus Crane. The breeding period for Sarus Crane was June to October. The clutch size was one or two eggs which were incubated by both the parents for about 30 to 31 days. The chalky white egg was about 11cm long and 6.5 cm wide (Plate 1). Some breeding pairs breed in September that facing the threats by many factors such as set up the fish net, habitat loss and natural enemies due to harvesting time. It may be assumed that, habitat quality often plays a major role until the fledging stage of the juvenile and adult Sarus Crane. Evaluating the breeding status of Sarus Cranes by Mayfield (1979) method, hatching (80.1%), crude breeding success (55.7%) and fledging success (69.6%) were calculated.

Habitat utilization of Sarus Cranes in non -breeding season and breeding season

During the non-breeding season, Sarus Cranes' seasonal movements were still need to some observations in Ayeyarwady Region. After breeding period of Sarus Cranes, they reunited their group. Breeding pairs with their chicks and non- breeding of Sarus Cranes used variety of wetland habitats such as wet marshes of agricultural land, cultivated land in shallow water (after harvesting), and reed bed mingled with lotus plant. According to the survey result, some breeding pairs used their breeding ground about three months with their chicks till fledging time. In these habitats, water were dried up and drained outside as well by farmer for seasonal crop planting. Farmers were ploughed for another planting on the breeding ground while Crane family foraged in these habitats. They eat snails, crustaceans, tuber roots and some invertebrates. According to field and interview survey, some pairs and other flocks (group were consisted up to thirty individuals) used wetlands and however they have a low preference for dry habitats even in non-breeding season. They moved to wet habitats from breeding ground where can forage and roost. According to the seasons and sites, it may be supposed that food sources were varies, especially foraging in complex habitats with water and the highest biodiversity value.

During the breeding season, breeding pairs and non-breeding pairs used different wetland sites. Foraging, nesting, and roosting in seasonal wetlands, natural wetlands, flooded paddy fields and lotus fields were observed. Sarus Cranes used various habitats which favorable for breeding process and would have territories. Nesting sites were most found in natural wetlands and flooded paddy fields. A mosaic of surrounding paddy fields serves the territorial requirements of the breeding pairs.

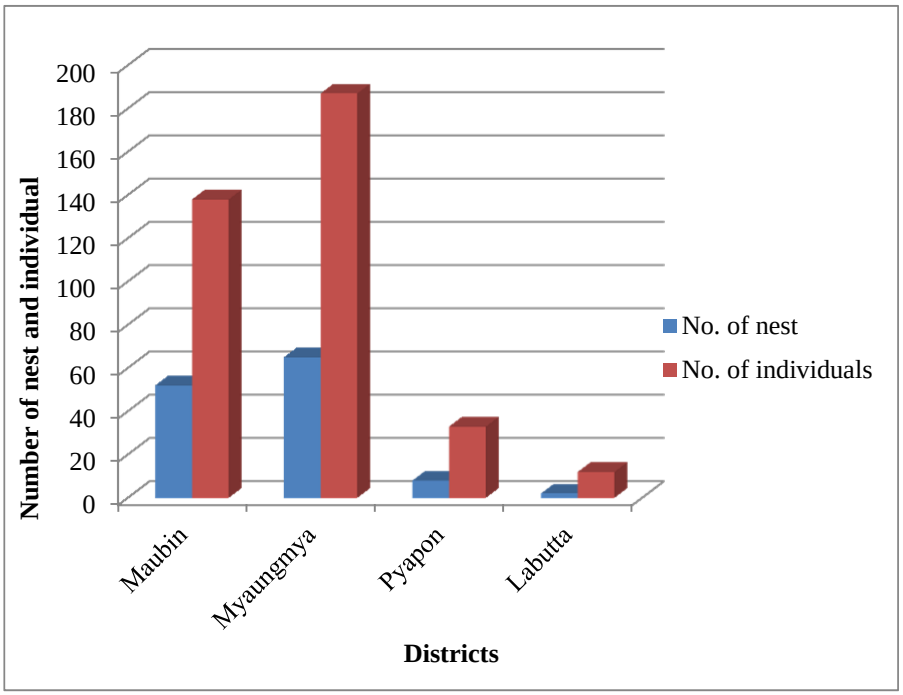

Figure1. Recorded number of nest and individual of Sarus Crane in four districts

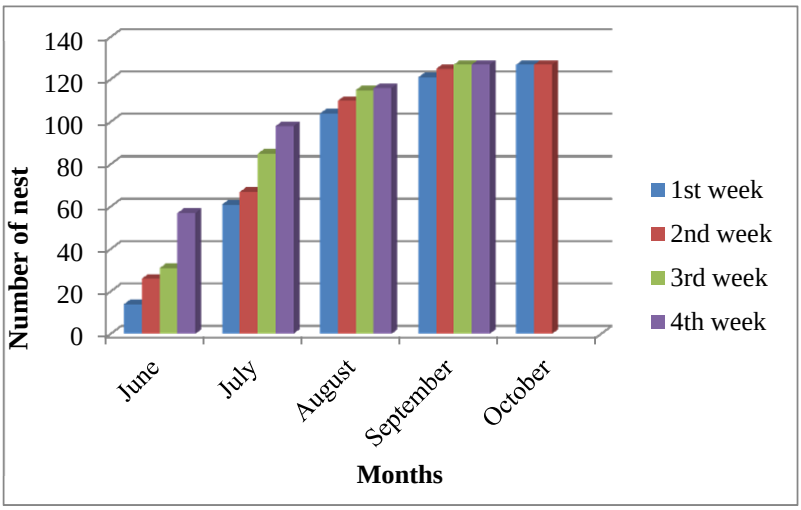

Figure2. Recorded number of nest in breeding period of Sarus Crane

Table 1. Recorded number of Sarus Crane nest and individuals in four Districts

Name of district	Number of township	Number of nest	Number of individual
Maubin	6	52	138
Myaungmya	5	67	187
Pyapon	2	8	33
Labutta	1	-	12
Total	14	127	395

A.Jumping of Sarus Crane

B. Dancing of Sarus Crane

C. Bowing movement of Sarus Crane

D. Sarus Crane' nest

E.Sarus Crane' eggs

F. Incubation of Sarus Crane

G. Swimming of Sarus Crane

H. 25 days old chick of Sarus Crane

I. A family of Sarus Crane

J. Hatching of Sarus Crane' egg

K. Sarus Crane' chick

L. Sarus Crane' chicks

Plate I. Recorded Sarus Crane photos

4. Conclusions

The expected outcomes of this research include a reliable estimate of the distribution, population, and documentation of the Cranes' habitats in Ayeyarwady Region. Scientific studies have identified the Ayeyarwady Region as one of the most important areas for Sarus Cranes in Myanmar. This research will be essential for the conservation effort of the fauna and their habitats in Ayeyarwady Region.

Acknowledgements

We are deeply indebted to Dr Thida Lay Thwe, Professor (Head), Zoology Department, University of Yangon, who permitted to do this research. We would like to express our special thanks to Dr Tran Triet, Director of South East Asia Program, International Crane Foundation (ICF), USA for his invaluable guidance and funding throughout this research. Special thanks are due to U Tin Aung Tun, freelance conservationist for his invaluable technical supporting during the survey period.

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Passiveness toward Drinking Water in Coastal Bangladesh Case Study of Rainwater Harvesting

Shota Yamada[1]

Abstract

Rainwater is one of the best solutions to the drinking water crisis in coastal Bangladesh, as it is safe and preferred by villagers. However, rainwater harvesting tanks (RWHTs) have not been distributed widely in the area. This study aims to investigate why many villagers do not have RWHTs and the factors that contribute to spreading RWHTs in this region. To this end, a field survey was conducted in a coastal village in Shyamnagar Upazila. The study found that only 34 of the 239 households in the village had RWHTs. These households obtained RWHTs from NGOs, markets, and friends or relatives, or made them themselves, as they faced drinking water limitation and wanted to increase the amount of rainwater preserved. A total of 12 households with RWHTs reported that they would not have obtained RWHTs had they not been prompted by NGOs, and 46 households with no RWHTs stated that they lacked them because they had not been prompted. The study concluded that, although many villagers experience drinking water shortages, they remain passive, awaiting support from development agencies. The study also found that currently, only households who are financially stable could benefit from the development aid related to drinking water.

Keywords: development activities, sustainable development, salinity, NGO, aid dependency

1. Introduction

Surface and ground water are affected by saline intrusion in coastal Bangladesh [4] [8] due to the prevalent natural, socioeconomic, and political systems [7]. Therefore, this region faces a scarcity of drinking water, and rainwater is, in many cases, the only source and solution of choice for many villagers of drinking water. The harvesting technology is easy to utilize [3] and coastal population prefer to intake rainwater [9]. Moreover, rainwater is safe enough to drink [5] [6] when collected and stored properly. However, it is often not enough to meet a full year's drinking water needs [1] because it cannot be collected in the dry season, as annual average rainfall ranges from 1,429 to 4,338 millimeters depending on the region, with most of it occurring during the monsoon season (from July to October) [2]. Nevertheless, as few alternative sources of potable water are available, concrete or plastic rainwater harvesting tanks (hereinafter: RWHTs) have been distributed in the area through markets and development agencies, such as governmental agencies, non-governmental organizations (NGOs), and social enterprises. However, many households still do not have RWHTs and are struggling to meet their annual drinking water needs.

The purpose of this study was to investigate the reasons why many villagers do not have RWHTs, as well as the factors that contribute to spreading RWHTs in this region. This study contributes to knowledge of development activities related to drinking water and, by extension, to solving the drinking water crisis in the area.

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2. Methods

A field survey was conducted in a coastal hamlet (total 239 households) in the Shyamnagar Upazila of Satkhira District from April 1 to May 6, 2019. This hamlet was chosen because it faces a serious drinking water shortage, most notably caused by saline intrusion, and development agencies have distributed RWHTs to mitigate it. Moreover, villagers have knew RWHTs made by plastic and cement not only through the activities and projects of development agencies but also through markets that sell them. The survey included the 34 households that owned RWHTs (hereinafter "tank holders"), as well as 51 randomly sampled households that did not have RWHTs (hereinafter "non-tank holders"). Participants were asked questions related to their incomes, the reasons that they had or had not obtained RWHTs, the places where they had obtained them or the agencies that had provided them, and the prices that they had paid for them. As the author do not have adequate knowledge of Bengali, the interviews were conducted with the help of an English translator.

3. Results and Discussion

The survey revealed that 38 (74.5%) of the 51 non-tank holders harvested rainwater using pitchers and other vessels, such as kolshi and motka (vessels traditionally used in Bangladesh), as well as plastic pots. Although they collected rainwater during the rainy season (roughly from June to September), they could not store enough for the rest of the year due to the insufficient capacity of the pots. Regarding the tank holders, 29 (85.3%) of the 34 households reported facing the same issue before they obtained RWHTs but 17 (50.0%) of the households reported annual self-sufficiency after obtaining RWHTs.

The tank holders had either obtained RWHTs from NGOs, markets, and friends or relatives or made them themselves (Table 1). Three households had more than one RWHT; two households had two, one from NGOs and the other homemade; and one household had three, one from a market, another from relatives, and the other homemade.

Table 1. Numbers of Rainwater Harvesting Tanks, Tank Holders, and Tank Providers

	NGO	Market	Relatives or Friends	Homemade	Previous Three*	Total
Numbers of Tank Holders	18	10	4	1	1	34
Numbers of Rainwater Harvesting Tanks	19	10	4	2	3	38

* Market, relatives or friends, and homemade.

NGOs provided RWHTs to 18 (52.9%) of the tank holders. Although a few tank holders had obtained RWHTs from NGOs of their own accord, NGOs generally had been prompting villagers to obtain them. Although several NGOs had been running RWHT distribution projects in the study hamlet, two were particularly active; one sold plastic RWHTs on monthly instalments through microfinance groups that it was operating, and the other sold cement RWHTs through finding beneficiaries in the hamlet.

A total of 10 (29.4%) of the tank holders had bought RWHTs at the markets. Although RWHTs were available at several markets, most of the tank holders had purchased them at the main market of Upazila, 16 kilometers from the study hamlet.

A total of 4 (11.8%) of the tank holders had obtained second-hand RWHTs from relatives or friends who had gained access to new drinking water sources. One household (2.9%) had made its own RWHT. Its owners reported that they had built it because they were envious of others who had RWHTs.

Although RWHT prices varied depending on the distributor, they generally correlated with their storage capacity. Plastic 1,000-liter RWHTs were sold by an NGO for 8,000 BDT (1 BDT = 0.0118581 USD), but RWHTs of the same capacity were sold in markets for 7,000 BDT. The difference of 1,000 BDT seemed to constitute interest on monthly payments. The net price of cement RWHTs was higher than that of plastic ones, but the NGO sold them at a more affordable price than the plastic ones by covering part of the cost. Therefore, cement RWHTs were sold 3,000 BDT for 3,200-liter RWHTs built above ground and around 5,000 BDT for 12,000-liter underground RWHTs by NGOs. Generally, the price was not significant; however, as some tank holders did not clearly remember it, and some RWHTs had been sold for a discounted price, as tank sellers and tank holders were on good terms.

The majority of the non-tank holders (36 households, or 70.6%) stated that they had not obtained RWHTs from NGOs or markets because they could not afford them. They particularly cited the absence of a monthly payment system in markets and the extra 1,000 BDT that NGOs charged as interest on monthly payments. In order to determine whether the relation between ownership and non-ownership of RWHTs and average annual income is statistically significant, an unpaired t-test was performed using Excel 2016 at a significance level of $p < 0.05$ (Table 2). The result showed that $t\ (36) = -3.32$ and $p = 0.002$, which clearly indicates that RWHTs were owned by households with sufficient money.

Table2. Unpaired t-test Result for Ownership of Rainwater Harvesting Tanks and Annual Income

	Numbers of Households	Annual Income (BDT)	t Value	Statistical Degrees of Freedom	p Value
Tank Holders	34	201,888	-3.32	36	0.002*
Non-tank holders	51	64,626			

* $p<0.05$

A majority of the households that had obtained RWHTs from NGOs (18 households, or 66.7%) reported that they would not have obtained them had they not been prompted by the NGOs. In addition, 46 (90.6%) of the non-tank holders stated that they did not have RWHTs because they had not been prompted by NGOs to buy them. In order to determine whether the relation between households prompted and households not prompted to buy RWHTs from an NGO and average annual income is statistically significant, an unpaired t-test was performed using Excel 2016 at a significance level of $p < 0.05$ (Table 3). The result showed that $t\ (20) = 2.15$ and $p = 0.04$, which clearly indicates that NGOs had prompted households with sufficient money to buy RWHTs.

Table3. Unpaired t-test Result for Prompts to Buy Rainwater Harvesting Tanks from NGOs and Annual Income

	Numbers of Households	Annual Income (BDT)	t Value	Statistical Degrees of Freedom	p Value
Households Prompted to Buy Rainwater Harvesting Tanks	20	197,495	2.15	20	0.04*
Households Not Prompted to Buy Rainwater Harvesting Tanks	49	64,846			

* $p<0.05$

Above all, this study concluded that villagers in coastal Bangladesh are passive toward issues related to collecting drinking water by obtaining RWHTs even though they are aware of the drinking water crisis, mainly caused by saline intrusion. In other words, they await support from development agencies, and they only consider obtaining RWHTs when they are targeted by aid projects. As a large number and a wide variety of aid projects has been carried out in Bangladesh, a certain mind-set has taken root among villagers that predisposes them to expect everything, even such a basic need as water, from development agencies.

This study, however, found that only financially stable villagers could benefit from aid related to drinking water. This means that poor villagers are largely excluded from the development assistance due to the monthly payment system that NGOs have introduced for the goods that they sell in order to both sustain their projects and generate a sense of ownership among villagers.

The findings of this study suggest that agencies should make efforts to change the villagers' passive attitude. At the same time, they should do more to help, especially the poorer villagers, reducing the monthly payment amounts and the interest rate or donating RWHTs with free cost.

4. Conclusions

This study focused on a coastal village in Bangladesh to investigate why, amid a serious drinking water crisis, some villagers own RWHTs, but many do not, as well as what factors contribute to spreading RWHTs in this region. Although villagers are aware of the drinking water crisis and have been harvesting rainwater using vessels of limited capacity, they are rather passive in terms of making efforts to obtain RWHTs, awaiting support from development agencies. This is due to a mind-set of aid dependency among villagers. Moreover, the poorer villagers are excluded from water-related development aid from NGOs, as they cannot afford the monthly instalments and the extra costs of purchasing RWHTs from them. It is, therefore, essential for NGOs to reduce the monthly payment amounts or donate RWHTs so that they can make RWHTs more affordable. Development agencies should also take notice of the villagers' passive attitude and adapt their strategies with an eye toward changing them so that villagers can do more to help themselves.

Acknowledgements

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Production of Starch Based Bioplastic from Cassava Reinforced with Microcrystalline Cellulose Using Sorbitol as Plasticizer

Yin Min[1], Naw Eh Htoo Phaw[2]

Abstract

The production of starch based bioplastics from cassava starch reinforced with microcrystalline cellulose (MCC) using sorbitol as plasticizer was investigated. Physical properties of bioplastics such as density, water uptake and tensile strength were determined. Bioplastics were prepared from cassava starch plasticized using sorbitol with variation of 20;25;30%(w/v of sorbitol to starch) reinforced with MCC fillers with range of 0 to 6% (w/w of MCC to starch). The results showed that the tensile strength was improved significantly with the addition of MCC but the elongation at break, density and water uptake of bioplastic film were decreased. The maximum tensile strength obtained was 3.0 MPa on the addition of MCC by 6% and sorbitol by 25%. Based on the data of FTIR, the produced film plastic did not change the functional group and it can be concluded that the interaction in film plastic produced was only a physical interaction. Surface morphologies of the bioplastic films were examined by scanning electron microscope (SEM).The prepared films resist to water and almost acid and alkali. With good adhesion between MCC and starch, the production of bioplastics could be widely used as a substitute for conventional plastics with more benefits to the environment.

Keywords: Bioplastic, tensile strength, elongation at break, microcrystalline cellulose, sorbitol

1. Introduction

As well known, synthetic polymer materials have been widely used in every field of human activity during last decades [1]. The major sources of land pollution include plastics, metal and glass containers, food wrapping, old furniture, garbage etc .Consumers today are the more concerned about the effects of petroleum based synthetic packaging film on the environment rather than their benefits due to their poor biodegradability, sustainability and biocompatibility. Replacing synthetic polymers with biopolymers is one alternative to overcome these problems. In resent year, biodegradable films have received great interest from the research community as they offer several advantages such as good biodegradability, biocompatibility, environmental friendliness, availability and even edibility [2]. Starch has attracted attention as a suitable material for the production of biodegradable plastics due to its natural abundance and low cost. Starch is mainly composed of two homopolymers of D-glucose [3]. Amylose, a mostly linear α-D (1, 4')-glucan and branched amylopectin, having the same backbone structure as amylose but with many α-1, 6'-linked branch points. To improve starch-based plastic characteristics and the mechanical resistance, many researchers have demonstrated the interest of using filler as reinforcement in thermoplastic matrixes and have shown that fibers incorporation can increase films tensile strength and elasticity modulus and decreases their elongation capacity [4]. Inorganic fillers such as glass fibers and ceramics have traditionally been used as the reinforcing filler for composites. However, recently, the use of organic fillers, such as lignocellulose based fibers, has been emerging into the market, as they give advantages over

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traditional inorganic fillers such as being low-cost, light weight, renewable, abundantly available, and biodegradable[5]. Reinforcing fibers such as cellulose has proven to be the most promising material. Microcrystalline cellulose used as reinforcement filler for starch based edible films was analyzed by Psomaidou et al (1996).MCC offers higher density of hydroxyl groups on its surface that is available for hydrogen bonding. [6]Biopolymer films need a plasticizer to increase the intermolecular spacing and molecular mobility of the polymer chains. This will increase the flexibility and stretch ability of biopolymer films [7].Without the plasticizers, biodegradable films are brittle due to extensive intermolecular force involving polymer chain-to-chain interactions [8]. The ability of a plasticizer to form a good biopolymer film depends on its compatibility with biopolymers, and appropriate amount of plasticizer as well as its number of free hydroxyl groups. Main non-volatile plasticizers are glycerol and sorbitol [9]. Apart from glycerol and sorbitol, water is a ubiquitous natural diluent, which can act as a plasticizer. Plasticizers are absorbed by starch granules and form hydrogen bonds with amylose and non-ordered amylopectin. This facilitates increased amylose and amylopectin mobility in the amorphous regions. Amylose and amylopectin rearrange, forming new intermolecular interactions.

Scientific Classification of Cassava

Family	Euphorbiaceae
Genus	*Manihot*
Species	*M. esculenta*
Botanical Name	*Manihot esculenta* L.
English Name	Cassava
Myanmar Name	Pi-law-pi-num

Figure1. Photograph of Cassava

2. Methods

The present research was carried out in the Chemistry Laboratory, Department of Chemistry, East Yangon University. Cassava sample was purchased from Myoma market, Thanlyin Township, Yangon Region. The collected samples were washed under running tap water followed by washing with distilled water. Chemicals used in this experiment were obtained from Sigma Company Ltd. The roots of cassava were washed, peeled and cut into small pieces and blended with blender. The resulting paste was mixed with water and the solution was filtered on a clean cotton cloth. The filtrate was then allowed to stand for 6 hours followed by the removal of the supernatant. The white precipitate (starch) was then recovered, air-dried and kept in the desiccator until constant weight and stored in polyethylene bags at room temperature.

In the preparation of bioplastic film, 50 g of starch was dispersed in 200 mL of distilled water in a beaker and 40 mL of sorbitol was added. The solution obtained was 0 % wt/wt of microcrystalline cellulose to starch and 20% wt/v of plasticizer to starch. In this way, 50 mL and 60mL sorbitol was using to get 25% and 30% wt/v of plasticizer to starch solution. For the preparation of 2%, 4% and 6% wt/wt of microcrystalline cellulose to starch, 1, 2, 3 g of microcrystalline cellulose was added in the above solution, respectively[7].Then the solution was placed into ultrasonic homogenizer tank and processed for about 50 minutes. After ultra-sonication, solution was removed from the tank. 5 g of starch was added to the solution and

heated using hot plate while stirred until become gelatinized (70 ºC). After mixing, the solution was cast onto flat and dried with 60 ºC for 24 hours. Once set, bioplastic was cooled to ambient temperature before peeled off the flat.

Figure 2. Step by step preparation of bioplastic

Water absorption of bioplastic was investigated by cutting film with size approximately 2×2 cm and then weighed. Bioplastic film was put into a container filled with distilled water for 24 hours. After immersion in water, film was removed from the water and then, they were dabbed with tissue paper to remove the water from the surface. Each weighing was done in triplicate in order to ensure results.

Mechanical properties of bioplastic such as tensile strength and elongation at break were examined based on the standard method ASTM D638-02A 2002 and ASTM D972-911991, respectively at Rubber Research Development Centre, Ministry of Agriculture. Functional group contains in bioplastic was measured by FTIR and morphology of prepared bioplastic by SEM. Solubility of prepared bioplastic in different solvents such as hydrochloric acid, nitric acid , sulphuric acid, ethanoic acid , sodium hydroxide, and ammonium hydroxide were tested. In this study, cutting film with size approximately 1×1 cm and then weighed. Bioplastic film was put into a container filled with 0.1M of 25 mL of different solvents for one hour. After immersion, film was checked whether the film was soluble or insoluble in the solvent. In biodegradability test, 1.1 g of pure starch prepared bioplastic sample (untreated) and maximum tensile strength of treated prepared bio-plastic sample was placed under the 1L beaker containing soil at a depth of 5 cm from the surface. Some amount of water was sprinkled on the soil to get bacterial enzymatic activity. These samples were weighed at each 3 days of interval from the beaker until disappear of the sample. Each weighing was done in triplicate in order to ensure results.

3. Results and Discussion

The yield of cassava flour extracted from cassava root was 38.31% on the basic of dry weight. Water absorption test is very important to determine the water absorptivity of material. Table 1 shows the water absorption of prepared bioplastic. In this study, films were emerged in water to verity the effect of plasticizer and concentration in the hydrophilic nature of starch based film. Water diffuses into the network chains of the film, thus, causing the film to swell. The swelling of all the films were rapid during the initial 6 hours and later followed by slower swelling rate until they reach equilibrium. Water uptake increase without MCC content this may be due to the fact that starch was more sensitive to water because of its hydrophilic nature than the cellulose.

Table1. Water Absorption of Prepared Bioplastic

Plasticizer (sorbitol) (w/v)%	Water uptake of prepared bioplastic film(%)			
	Reinforcement (Microcrystalline cellulose) (w/w) %			
	0	2	4	6
20	43.41±0.095	37.00±0.100	35.06±0.081	31.71±0.115
25	55.35±0.045	42.96±0.105	32.89±0.075	22.76±0.095
30	34.14±0.082	35.18±0.075	31.23±0.096	28.90±0.284

In order to know the quality of resulted bioplastic film, several parameters which usually used to analyze resulted bioplastic are measured such as tensile strength, elongation at break. The effect of addition of microcrystalline cellulose and sorbitol depend on the tensile strength of prepared bioplastic were shown in Table 2 and Figure 3. The tensile strength of prepared films was reasonably good. In this study, tensile strength of sorbitol plasticized film was necessary of reinforcement like microcrystalline cellulose. The addition of MCC as reinforcement fillers represented an improvement in tensile strength of sorbitol plasticized film. Thus bioplastic with MCC content 6 % and 25 % sorbitol provided the maximum tensile strength for 3.0MPa. This may be due to the fact that the addition of MCC on gelatinized starch films resulted in grouping of intermolecular hydrogen bonding.

Table 2. Tensile strength of Prepared Bioplastic

Plasticizer (sorbitol) (w/v)%	Tensile strength of prepared bioplastic film(MPa)			
	Reinforcement (Microcrystalline cellulose) (w/w) %			
	0	2	4	6
20	0.23	0.62	0.81	2.5
25	0.54	0.70	0.92	3.0
30	0.50	1.10	1.20	2.20

Contrary to tensile strength value, the result of elongation at break decreased as the addition of MCC content as shown in Figure 4. Results showed a decrease in elongation at break with the increasing MCC content from 0 to 6 %. It may be possible due to hydrogen bonding between hydroxyl group (O-H) from starch and hydroxyl and carboxyl group (COOH) of cellulose as a result of higher strength and lower elongating at break. Moreover, the addition of plasticizer to bioplastic had decrease of elongation at break. Bioplastic with MCC content 0% and 20% wt/v of sorbitol provided the maximum elongation at break value for 73%. The observed increase in film elongation is plasticizer decrease because the intermolecular bond of starch matrix was substitute instead of hydrogen bonds formed between plasticizer to starch molecule. In this study, the role of decreasing reinforcement is more potent than increasing plasticizer on maximum elongation at break.

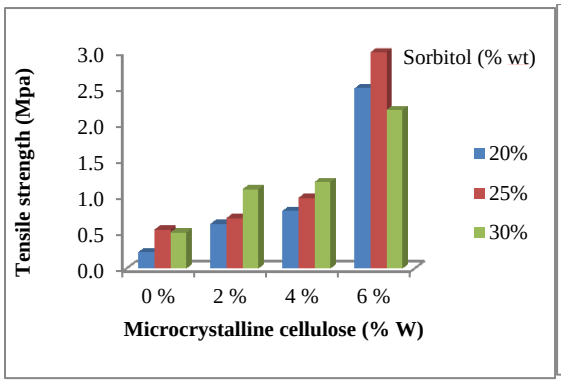

Figure 3. Tensile strength of sorbitol- plasticized film

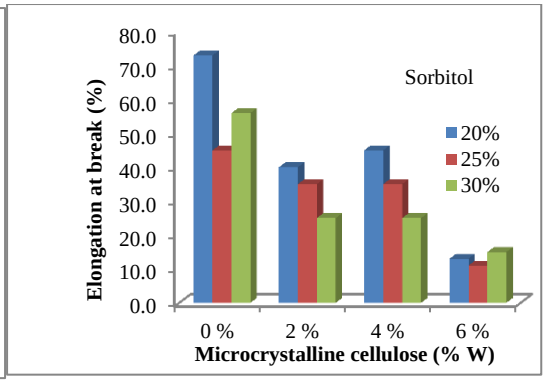

Figure 4. Elongation at break of sorbitol- plasticized film

The interaction between bioplastic components can be identified from FT-IR. The analysis of FT-IR showed the characteristics peaks concerned with bioplastics. If components from immiscible blends, there is no appreciable changes can be found in FT-IR spectra. However, if the components are compatible, there is chemical interaction between their chains culminating in the change of FT-IR spectra. Figure5 showed the results of characteristics of peak for cassava starch and pure starch bioplastics, the green line represented characteristic peak of cassava starch. Pink line showed characteristic peaks for pure starch bioplastic film. Figure6 described the results of characteristics of peak of microcrystalline cellulose. Figure 7 represented the results of characteristics of peak of starch/MCC/sorbitol bioplastic and starch/ sorbitol bioplastic .The pale blue line represented starch/MCC/sorbitol bioplastic characteristic peaks and the red line represented starch/sorbitol bioplastic characteristic peaks. From those figures could be seen that characteristics peaks of bioplastic without adding MCC and plastic with adding sorbitol have the same functional groups and no new cluster formed. The spectra of bioplastic film produced from this study displayed the presence of four major absorption peaks which are O-H stretching, C-H stretching, C=O stretching and C-O stretching and described in Table 3.

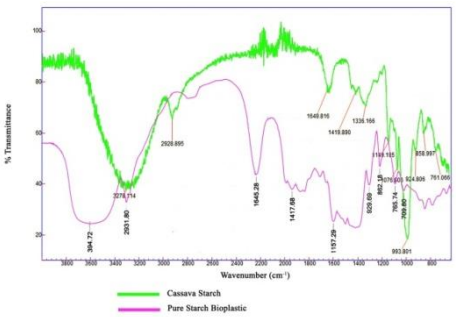

Figure 5. FT IR spectra of cassava starch and pure starch bioplastic

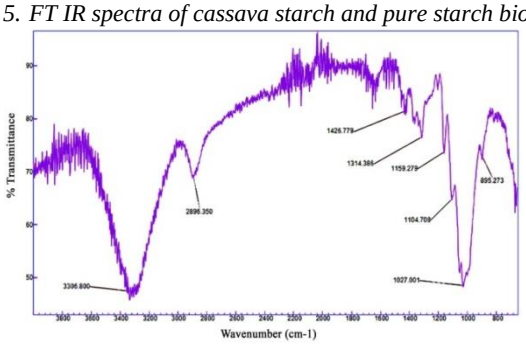

Figure 6. FT IR spectra of microcrystalline cellulose

Figure 8 showed the morphology of prepared bioplastic film. Morphology of bioplastic film produced is closely related to the concentration of sorbitol and microcrystalline cellulose that were added in synthesizing of the film plastic produced. Visually one of the results of optimum condition of film plastic produced is not porous and it looks no cracks, the film appearance is transparent. The morphology structure of prepared bioplastic film has not given homogeneous morphology structure.

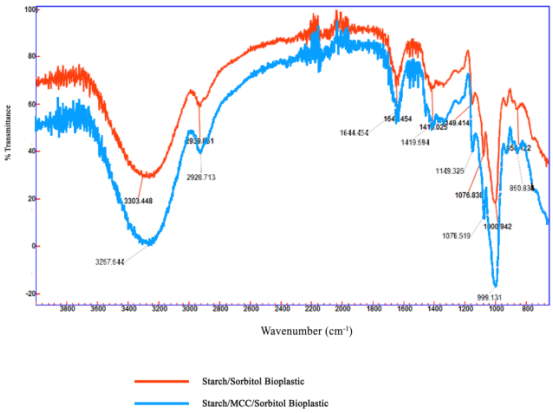

Figure 7. FT IR spectra of starch/MCC/sorbitol bioplastic

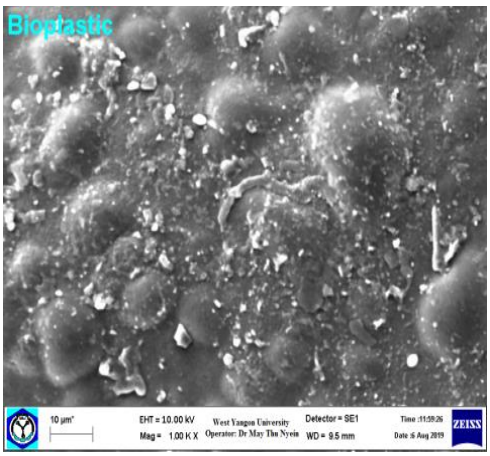

Figure 8. SEM image of prepared bioplastic film and starch/sorbitol bioplastic

Table 3: The main FTIR absorption peaks of cassava starch and pure starch bioplastic, microcrystalline cellulose and starch/MCC/sorbitol bioplastic and starch/sorbitol bioplastic

Functional group	Wave Number (cm^{-1})	
	Observed value	Literature value
O-H stretching	3373.04	3600-3300
C-H stretching of CH_2 group	2931.60	2904
C=O stretching of CHO group	1646.77	1637
C-O stretching of carbonyl group	1026.98	1149

The solubility of prepared bioplastic film in different acid and alkali was tested and the result was described in Table 4 and Figure 9. After one hour, bioplastic film was insoluble in hydrochloric acid, ,nitric acid and ammonium hydroxide solution however, it was partially soluble in sulphuric and, ethanoic acid and sodium hydroxide

Table 4: The solubility of acid and alkaline test for prepared bioplastic film

Solvent used	**Property**	**Partially**	**Completely**	**Insoluble**
Hydrochloric	Acid test			✓
Sulphuric acid	Acid test	✓		
Nitric acid	Acid test			✓
Ethanoic acid	Acid test	✓		
Sodium	Alkaline test	✓		
Ammonium	Alkaline test			✓

The weight loss experienced by starch –based bioplastics in two weeks soil burial test was described in Table 5 and Figure 10. The weight loss of plastic sheets during burial in soil indicates the amount of degradation in natural environment by action of microorganisms. The starch content consumed by soil microorganisms will fracture the polymer chain thus cause the biodegradation. It was found that bioplastics with un-filler sample was rapidly degraded than that of filler sample. So current research were benefits in findings the possible solution to the environmental tractability of biomaterials.

Figure 9.Solubility test of prepared bioplastic (a) initial test (b) and (c) after 1 hour

Table 5 Weight loss of Prepared Bioplastic

Bioplastic Film	Initial weight (g)	Weight(g)			
		after 3 days	after 6 days	after 9days	after 12days
Without MCC	1.1 ± 0.0004	0.5322 ± 0.0032	0.0258 ± 0.0001	0.031 ± 0.0005	Not observed
With MCC & sorbitol	1.1 ± 0.0003	0.7397 ± 0.0018	0.0815 ± 0.0016	0.0134 ± 0.0004	Not observed

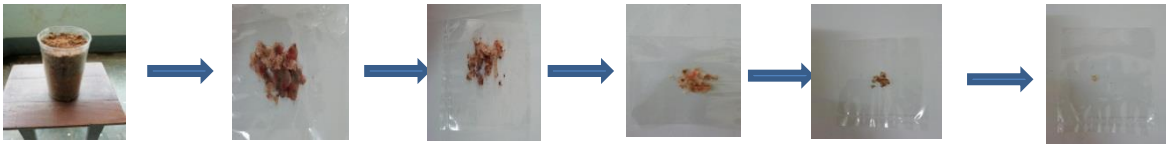

Figure 10. Biodegradability of prepared bioplastic film during 2 weeks

4. Conclusions

From this study, it can be concluded that the starch-based bioplastic has been successfully synthesized from cassava starch, MCC as reinforcing filler and sorbitol as plasticizer. Results of this study showed that the addition of MCC and sorbitol, some changes in mechanical properties of bioplastic film such as being transparent, clear, homogeneous, flexible, and easily handled. The optimum formulation composition has tensile strength value 3.0 MPa at concentration of MCC by 6 % and sorbitol by 25 %. The improvement in bioplastics with reinforcing MCC could be attributed to the strong hydrogen bond between hydroxyl groups of the interface of both MCC fillers and starch matrix. Density of bioplastics decreased as MCC content increased. Furthermore, the prepared bioplastic film was resistant to water, hydrochloric acid and ammonium hydroxide and it was partially soluble in ethanoic acid, sulphuric acid and sodium hydroxide. Based on the data of FTIR, the produced film plastic did not change the functional group and it can be concluded that the interaction in film plastic produced was only a physical interaction. The morphology structure of prepared bioplastic film has not given homogeneous morphology structure. These products have exceptionally high biodegradable properties with decent mechanical properties and make them a suitable alternative for the existing conventional plastics. Furthermore, starch is renewable resource, cheap and can be easily modified. With good adhesion between MCC and starch, the production of bioplastics could be widely used as a substitute for conventional plastics with more benefits to the environment. This means someday it is unnecessary to rely on petroleum to prepare polymers, people may ‘plant’ polymers of suitable performances from the earth, and the environmental problems will be no longer as severe as today.

Acknowledgements

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Study on Ion-exchange of Toxic Lead in a Model System by Metal-Humate Complexes

Naw Aye Aye Thein[1]

Abstract

The removal of Pb2+ ions was studied by using ion-exchange properties of metal-humates such as Fe-humate, Zn-humate and Mg-humate. Metal-humate complexes were prepared by using humic acids with Fe2+, Zn2+ and Mg2+ ions. For the removal of Pb2+ ion, the ion-exchange studies of different concentration of Pb2+ ions and metal-humates (Fe-humate, Zn-humate and Mg-humate) were carried out. The ion-exchange abilities depend on pH, initial concentration of Pb2+ ions, contact time, dosage of rnetal-humates and their ion-exchange capacities. The ion-exchange studies for the removal of Pb2+ ions with various metal-humates indicated that each humate possesses different binding affinity. The order of binding affinity of Pb2+ to metal-humates are Fe-humate > Zn-humate ~ Mg-humate .The removal of Pb2+ ions with Fe-humate, Zn-humate and Mg-humate ware characterized by EDXRF technique.

Keywords: humic acid, Fe-humate, Zn-humate, Mg-humate, Pb^{2+} ions

1. Introduction

Humic acid can be extracted from any material containing well decomposed organic matter – soil, coal, composts, etc. Extraction is by way of treatment of these materials with a solution of sodium hydroxide. This dissolves much of the organic matter present. Then take this solution and add enough acid to drop its pH-2 to about organic material will begin to flocculated and can be separated from the liquid portion, The flocculated material is humic acid and remains in solution is Fulvic acid.Humic acid is effective in removal of heavy metal ions[1]. Metals are bound to the carbon skeleton of humic substances through heteroatoms such as nitrogen, oxygen or sulphur. The cation exchange capacity of humic acid is 200-500 meq per 1100 grams. Depending on the physical state of the humic acid, the binding mechanism may be different. Binding is stronger with precipitate humic acid than with dissolved humic acid. Metals are bound to the carbon skeleton humic substances through heteroatom such as nitrogen, oxygen or sulphur. The most common metal binding occurs via carboxylic and phenolic oxygen, but nitrogen and sulphur also have a positive effect on metal binding [2].

2. Methods

2.1 Extraction of Humic Acid from Coal Sample and Preparation of Metal-Humate Complexes

In the present work, humic acid was extracted from coal sample by using different concentration of sodium hydroxide. Two fractions obtained after extraction materials and a supernatant consisting of a mixture of humic acid and fulvic acid .The separation of these two acids are done at pH 2 where precipitation of humic acid occurred. All fractions were separated by centrifugation and dried by vacuum oven at 70° C. The purified humic acid extracted from

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coal sample was dissolved in a 1M sodium hydroxide solution. Metal (II) solution was added to dissolve humic acid, which were acidified with concentrated sulphuric acid to pH 2 and allowed to standard for 24 hr. Metal- humate was separated by centrifugation and dried by vacuum oven at 70° C.

2.2 Ion-exchange study for the removal of Pb^{2+}ions by using Prepared Metal-humates

Effect of pH, Contacted Time and Dosage of Metal-humates

Fe-humate,Zn-humate and Mg-humate (1g) was treated with 100 mL of Pb^{2+}solution. The initial concentrations of Pb^{2+} solutions were varied in (200 to 1000) mgL^{-1}. The pH values (3 to 8) of the solution were adjusted by the addition of 0.1 M hydrochloric acid and 0.1 M sodium hydroxide. The contact time was varied at interval of 3 to 60 mins. The solution was shaken with a shaker at room temperature for 1 hr. Then, the mixture was filtered through a filter paper. Various amount of metal humate (1, 2, 3 and 4 g) were mixed with 100 mL of 1000 mgL^{-1} Pb^{2+} solution in each at optimum pH and then were shaken. After 60 min contact time, the mixture was separated by filtration. The residual content of Pb (II) ions in filtrate were determined by complexometric titration [4]

3. Results and Discussion

Characterization of extracted humic acid and Preparation of Metal-Humate Complexes

From EDXRF spectrum of extracted humic acid from coal sample was shown the presence of relative abundance of elements such as S, Si and Mo in extracted humic acid.The EDXRF spectra of Fe-humate,Zn-humate and Mg-humate indicated that the presence of relevant metals ions ($Fe^{2+,}Zn^{2+}$ and $Mg^{2+)}$ were chelated to humic acid. The results were shown in Figures 3.1,3.2, 3.3 and 3.4.

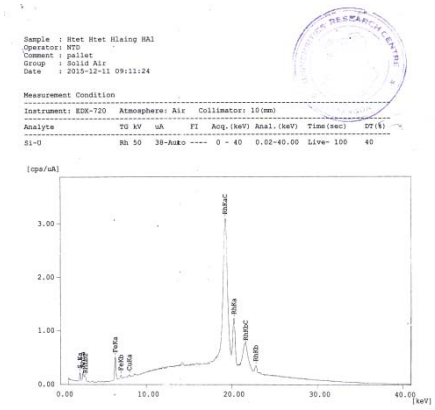

Figure 3.1. EDXRF spectrum of extracted humic acid

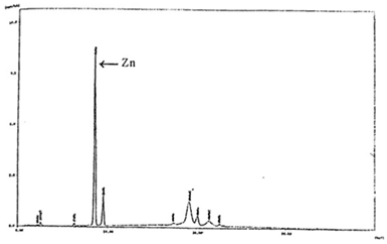

Figure 3.2. EDXRF spectrum of Fe-humate

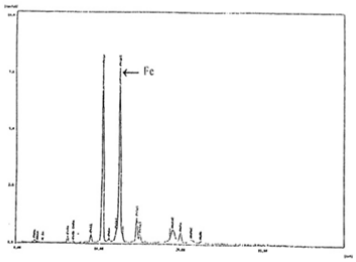

Figure 3.3. EDXRF spectrum of Zn-humate

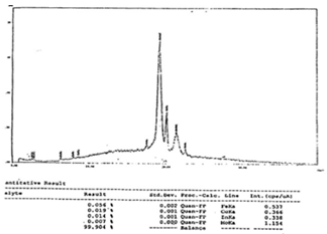

Figure 3.4. EDXRF spectrum of Mg-humate

3.2 Study on removal of Pb^{2+} Ions by using Prepared Metal-humates

The effect of pH on the removal of Pb^{2+} ions was investigated at three pH regions, i.e., at pH < 7, pH = 7 and pH > 7. Table 3.1 shows the percent removal of Pb^{2+} ions ranging from an initial concentration of 200 mg L^{-1} to 1000 Mg L^{-1} by iron-humate at different pH values (3, 4, 5, 6, 7 and 8). Figure 3.5 depicts the percent removal of Pb^{2+} by Fe-humate as a function of pH. As pH increases the percent removal also increases, until it reaches an optimum condition of pH 5. From the optimum pH of 5 onwards, the percent removal of Pb^{2+} ions began to decrease to very low values up to pH 8. The observation is not surprisingly because as a general rule ligands tend to form protonated ligands at low pH and metal ions tend to form hydroxyl complexes at high pH. Consequently, the reaction between the metal ion and the ligands is often most favorable at intermediate pH values.

Table 3.2 shows the percent removal of Pb^{2+} ions by zinc-humate at different pH value. The initial concentrations are ranging from 200 mgL^{-1} to 1000 mgL^{-1}. Figure 3.6 presents the percent removal of Pb^{2+} ion as a function of pH. It can be seen that the profiles relevant to lead % removal by znic-humate depends on pH. for each initial concentration, the percent removal of Pb^{2+} increases with increasing pH ranging from pH 3 to 6. The maximum percent removal is observed at pH 6. After pH 6, the percent removal remains steady. The sorption process of zinc-humates follows a similar trend as iron-humate.

Table 3.3 shows the influence of pH on the removal of Pb^{2+} ions by magnesium-humate using initial concentrations of 200 mg L^{-1} to 1000 mg L^{-1} at different pH values. Figure 3.24 depicts the profiles of the removal of Pb^{2+} ions and as a function of pH. In view of the profiles of the curves, high percent removal corresponds to low concentration of Pb^{2+} and low percent removal as regard to high concentration of Pb^{2+}. The removal of Pb^{2+} by magnesium-humates is accompanied by complexation and ion-exchange. First at lower pH values up to 5, the process is

typically and adsorption process. At pH 5, maximum removal has taken place, but after pH 5, the percent removal remains steady.

This may be attributed to ion-exchange and some complexation taking place provably at the saturation time of 15 to 20 minutes. The major mechanism is ion-exchange between Pb^{2+} ion ions in solution and Fe^{2+}, Zn^{2+} and Mg^{2+} from humates. Table 3.4 and Figure 3.7 show the comparison of the effect of contact time on ion-exchange capacities of various metal-humate (Fe^{2+}, Zn^{2+} and Mg^{2+}) towards initial concentration of Pb^{2+} ions (C_0) of 200 mgL^{-1} at their optimal pH values. According to this Figure, within 10-20 minutes, maximum ion-exchange capacities are achieved. A two stage kinetic behavior is observed, i.e., a very rapid initial exchange over a few minutes, followed by a long period of much slower uptake. The order of magnitude with regard to ion-exchange capacity of metal-humates towards Pb^{2+} ions may be as follows: Fe-humate > Zn-humate ≈ Mg-humate.

Table 3.1 The effect of pH on the removal of Pb^{2+} ion by Fe-humate

Intial conc: of Pb^{2+} (mgL^{-1}) ↓ / pH →	*Percent removal of Pb^{2+} at different pH values*					
	3	4	5	6	7	8
200	35	55	78	74	64	50
400	33	52	75	60	67	45
600	30	48	73	58	54	43
800	25	40	70	64	52	38
1000	20	35	64.5	60	46	35

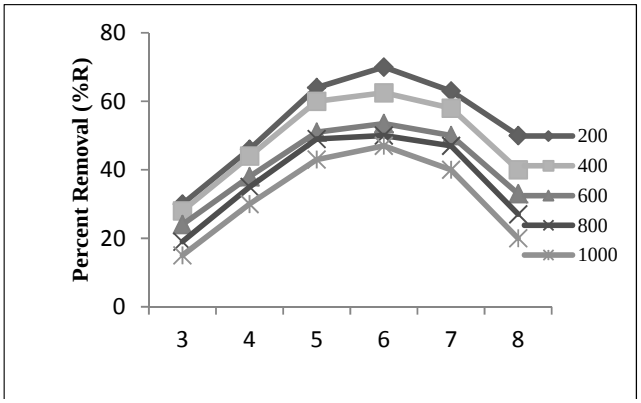

Figure. 3.5 The effect of pH on the removal of Pb^{2+} ions by Fe-humate

Dosage = 1g Fe-humate,

Volume of Pb^{2+} solution = 100 mL

Table 3.2 Effect of pH on the removal of Pb^{2+} ion by Zn-humate

Intial conc: of Pb^{2+} (mgL^{-1}) ↓ / pH →	Percent removal of Pb^{2+} at different pH values					
	3	4	5	6	7	8
200	30	46	64	70	63	50
400	28	44	60	62.5	58	40
600	24	38	51	53.5	50	33
800	19	35	49	50	47	27
1000	15	30	43	47	40	20

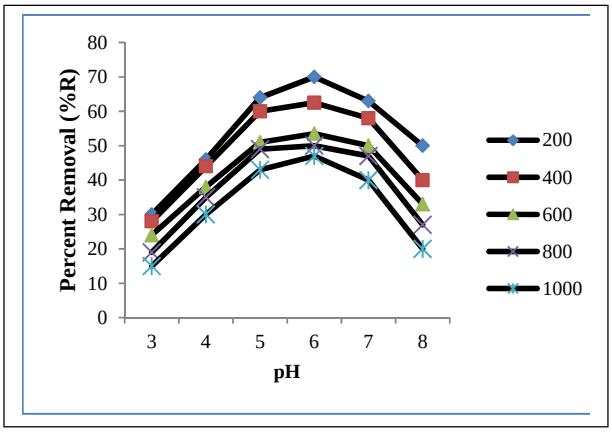

Figure 3.6. Effect of pH on the removal of Pb^{2+} ions by Zn-humate

Dosage= 1g Zn-humate,

Volume of Pb^{2+} solution = 100 mL

Table 3.3 The effect of pH on the removal of Pb^{2+} ion by Mg-humate

Intial conc: of Pb^{2+} (mgL^{-1}) ↓ / pH →	Percent removal of Pb^{2+} at different pH values					
	3	4	5	6	7	8
200	31	52	67.5	64	58	40
400	28	44	60	56	54	38
600	25	38	51.7	49	48	35
800	20	34	47	45	45	31
1000	17	30	45	42	40	25

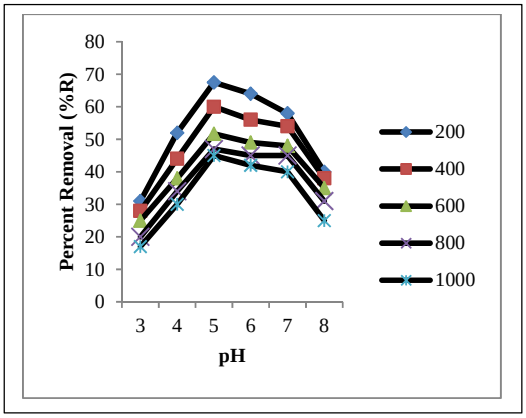

Figure 3.7 Effect of pH on the removal of Pb^{2+} ions by Mg-humate

Dosage= 1g Mg-humate,

Volume of Pb^{2+} solution = 100mL

Table 3.4 The Effect of contact time on the ion-exchange capacities of metal-humates towards Pb^{2+} ions

Metal Ions	pH	Ion-exchange Capacity ($mg\ g^{-1}$)									
		3*	6*	9*	12*	15*	20*	30*	40*	50*	60*
Fe^{2+}	5	5	8	11	13	15.5	15.5	15.5	15.5	15.5	15.5
Zn^{2+}	6	4	7	10	12	14	14	14	14	14	14
Mg^{2+}	5	3	6	9	11	13.5	13.5	13.5	13.5	13.5	13.5

*= Time (min) of reaction mixture

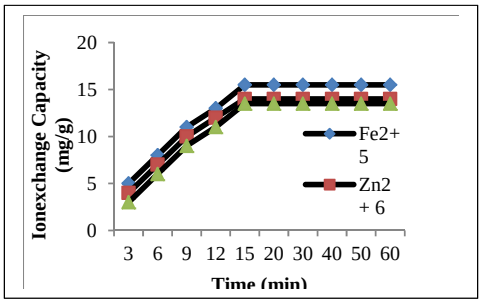

Figure 3.8The effect of contact time on the ion-exchange capacities of metal-humates towards Pb^{2+} ions

Dosage= 10 gL^{-1} of each metal-humate,

Initial concentration of Pb^{2+} = 200 mgL^{-1}

3.3 Characterization of Pb^{2+} on metal humates

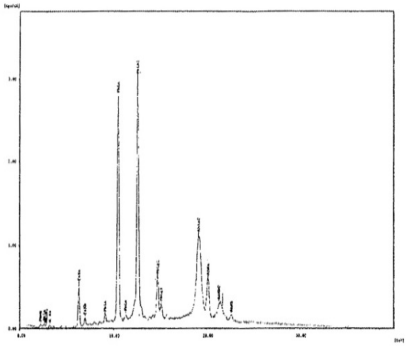

Figure 3.11EDXRF spectrum of lead on Mg-humate

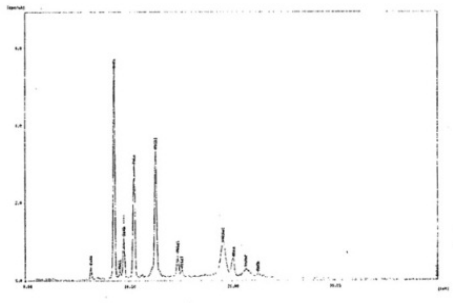

Figure 3.40 EDXRF spectrum of Lead on Zn-humate

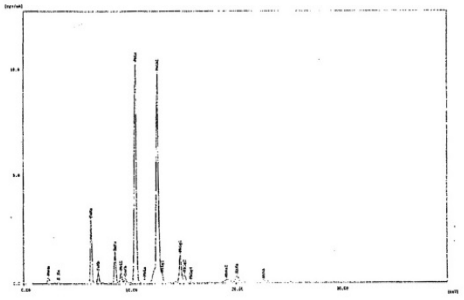

Table 3.5 Relative Abundance of the Elements

Elements	Relative Abundance (%)
Pb	82.737
Pb	78.980
Pb	71.282

4. Conclusion

The goal of this research work is to study the removal of toxic lead by ion-exchange with metal-humates prepared from extracted humic acids of coal sample. Ion-exchange studies for the removal of lead ions with iron-humate, zinc-humate and magnesium-humate indicated that each humate possesses different binding affinity relevant to pH, initial concentration, contact time and ion-exchange capacity.. The order of reducing lead toxicity by metal-humates were Fe-humate > Zn-humate ≈ Mg-humate. Hence, the findings of this work supplement the important of humic substances which have specific properties such as a high cation exchange capacity and the ability to chelate metal ions.

Acknowledgements

The author feel indebted to Professor and Head Dr Ni Ni Than, Department of Chemistry, University of Yangon, Yangon, Myanmar, for her stimulating suggestions.

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Modification and Enzyme Immobilization of Natural Cellulose Fiber (Polysaccharide)

Mya Mya Sainn[1] et al

Abstract

The coconut white meat from endosperm (after extracting coconut milk) is one of the waste products in Myanmar. It is very cheap and readily available. In this present work, the coconut white meat was selected for extraction of natural cellulose fiber. The aim of present work is to study the modification and the possibility of enzyme immobilization on natural cellulosic material by periodic acid .The natural cellulose fiber was extracted from coconut white meat powder (300 ~ 400μm) of mature endosperm by chemical method . The vicinal diol group of prepared cellulose fiber was oxidized to dialdehyde group by NaIO4 solution .The aldehyde groups formed were detected by different chemicals assays and confirmed by FT IR spectroscopy .The physical change of activated cellulose fiber were performed by SEM and optical-microscopic images. The soluble liapse enzyme was covalently link to dialdehyde group of modification cellulose fiber was tested by Schiff's base reaction. The enzyme immobilize cellulose fiber was then confirmed by nitrous acid test, FT IR spectroscopy and optical-microscopic images.

Keywords: cellulose fiber, immobilization, dialdehyde, Schiff's base

1. Introduction

Coconut white meat from endosperm (after extracting coconut milk) is one of the waste product in Myanmar. It is very cheap and is readily available. In the present work, germinating coconut endosperm as selected for extraction of lipase. It is consequently chosen as support material sources for enzyme immobilization. Coconut is an important commodity in many humid tropical regions of the world. It is also available and versatile in Myanmar. *Cocos nucifera* L. (coconut) palm is a member of the Arecaceae family, subfamily Arecoideae which is characterised by three called ovary drupaceous fruit with a woody or stony endocarp provided with three germ pores, while the fruit is usually one seeded .The coconut fruits still possess their outer layers, a smooth skin ranging colour from green to brown around a fibrous layer, which is used commercially as coir. These layers surround the hard-brown shell, which encases the coconut seed. The outer layer of the seed, the test, is the brown papery material that adheres to the white flesh (endosperm) when it is removed from the shell [1]. The high level of lipid, polysaccharide and high level of polyphenols were found in tissue of coconut [2]. Polysaccharides are carbohydrate which yield a large number of monosaccharide molecule on hydrolysis. The most widely spread polysaccharides have the general formula $(C_6 H_{10} O_5)_n$ eg. Starch, cellulose. Cellulose $(C_6 H_{10} O_5)_n$ is the main constituent of the cell wall of plants and also occur in certain animal tissues. It is the most widely distributed organic compound and it's main source in cotton (almost pure cellulose) and wood . It is a hydrophilic linear glucose polymer, a polyalcohol more or less uniformly composed of anhydroglucose units by 1, 4 glucosidic bonds. The linkages in cellulose cause the molecules to form hydorgen bonds. Cellulose is a white solid, insoluble in water but soluble in water but soluble ammoniacal

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copper hydroxide solution (Schwetzer's reagent). Fibrous polysaccharides support are very popular for enzyme immobilization because they contain hydroxyl groups that can be activated directly by introduction of an electrophilic group, reactive towards enzyme, into the support[3]. Fibrous supports include such desirable characteristics as ultra-high specific surface area, good chemical and thermal stability, hydrophilicity and insolubility [4].

Cellulose fibre were oxidized with sodium metaperiodate (10^-_4) at positions 2 and 3 of the anhydroglucose units to obtain diadehyde cellulose . The aldehyde groups of the dialdehyde cellulose were able to react with amino group of enzyme to form covalent bonds and resulted in a dialdehyde immobilized enzyme [5]. IO^-_4 oxidation is a highly specific reaction and widely used in the structure analysis of carbohydrates[6]. The aldehyde groups in the modified cellulose fiber can be detected by chemical means and spectrophotometric method. The functional aldehyds group can be introduced into a glycoprotein by oxidizing the carbohydrate moiety by periodate oxidation without significantly affecting the enzyme activity. The enzyme could then be covalently linked containing an alkyl amine group through the Schiff base[3]. Coconut white meat endosperm, after extracting coconut milk, is one of the cellulosic waste products. It is very cheap and is readily available in Myanmar. These fibrous materials offer versatile porosity and ultra high specific surface for superior enzyme binding and substrate accessibility. It is consequently chosen as material in the present study on support for enzyme immobilization [5]. Sodium metaperiodate treated cellulose fiber was used for immobilization of lipase by covalent binding via Shiffs base linkages.

Fresh seeds of coconut should be placed on damp peat with the broadest surface downwards at a temperature of 24- 27°C. The seed germinates slowly, taking up to 4 months before the shoot appears. During germination, the single cotyledon (seed leaf) grows inside the seed activity. Its sheath protects the seedling root and shoot and grows with them through the softest of the three eyes at the base of the shell. Part of the cotyledon, the coconut apple, enlarges -into the cavity inside the coconut and absorbs nutrients from the endosperm and coconut water. The content of lipase was found to be the highest level in this stage. Because the seedling has accessed to the food source, it can grow for some time before making contact with the soil [7].Lipase (Triacylglycerol acyl hydrolase (E.C 3.1.1.3)) was extracted from germinating coconut endosperm by acetone powder method[8] and partially purified by ammonium sulphate [9].

2. Methods

Preparation of Cellulose Fiber

The seeds of coconut *(Cocos nucifera* Linn.) were collected from North. Oukalapa market. The endosperm of matured coconut seeds were cut into small pieces. The pieces were ground by blender which was kept chilled to prevent fermentation. First, coconut milk was squeezed out and the residue was washed three times with distilled water. Then the purified coconut white meat was dried in air oven at 40 - 60°C. The dried coconut white meat (200g) was defatted with Petroleum ether by the Soxhlet extractor. After complete extraction of oil, the defatted white meat sample was removed from the extractor and air dried at room temperature. The defatted coconut white meat was boiled three times with 1.5 L of distilled water for 1 hour and dried in air oven .The sample was purified by boiling

in 500 ml of 1.25% sulphuric acid and 500 ml of 1.25% sodium hydroxide for 1 hour followed by washing with 500 ml of distilled water and 250 ml of ethanol. Then, it was dried in hot air oven at 100˚ C for 3 hours. The purified samples were stored in an air-tight container to prevent moisture changes and other contamination.

Preparation of Modified Cellulsoe Fiber by 2% Periodic Method

For immobilization of Lipase enzyme, coconut fibre was pretreated with 2% periodic acid. A 10 g (oven dried weight) purified cellulose fiber was weighed into 250 ml conical flask and presoaked with 2% w/w sodium metaperiodate solution. The conical flask was then and mixed for 2 hours in the dark place. At the end of reaction, the fibers were carefully washed with 50 ml of ethylene glycol and washed with 50 ml methanol. The coconut fiber was then neutralized by repeated washing with deionized water.

Characterization of Modified Cellulose Fibre by Qualitative Chemical Assay

Qualitative chemical assay was carried out according to the standard procedure. Investigation on the color changes and precipitation, it should be known whether a particular class of aldehyde group was present or not. The aldehyde group in modified cellulose fiber were qualitatively confirmed by the positive reaction of chemical assay (2, 4-DNP, Benedict, Tollen, Fheling, Schiffs, and Benzedine) .

Immobilization of Liapse on Modified Coconut Fiber

1.0 g sample of dried oxidized cellulose fiber and standard cellulose was separately immersed in 30 ml of purified lipase (0.4 g enzyme in 10 ml of pH (5.5) citrate-phosphate buffer) and constant shaking with 150 rpm for 30 mins at room temperature. Then the two types of cellulose fiber were individually filtered to remove excess enzyme and followed by washing with 800 ml of distilled water. Next, the unimmobilized enzyme on supporting material was washed again with 100 ml of distilled water till the enzyme activity in washed water was found to be absent. The immobilized lipase on modified coconut cellulose fiber and on cellulose (BDH) was kept at 4°C.

Characterization of Lipase Immobilized Cellulose Fiber by Chemical Assay

0.2 g of immobilized and modified fiber was separately placed in the test tubes. Equal amount of H_20 and concentrated HCI were added to each test tube. And then the test tube was cold in ice and 10 drops of 10% $NaNO_2$ (0°C) was added and cold to 0˚C for 15 mins. Finally, 2-4 drops of 20% NaOH was added to the cold mixture. The result of reaction was observed and recorded .

Characterization of Various Cellulose Fiber and Immobilized Fiber

The Fourier transform infrared spectra of pure coconut fiber, modified cellulose fiber and immobilized cellulose fiber were recorded on Fourier transform infrared spectrometer, FTIR 8400 Shimadzu Hyper IR (Japan). The natural coconut fiber and modified cellulose fiber were analyzed by SEM (Scanning Election Microscope). The resultant Micrographs were presented in Figure (4).The samples were examined under optical microscope (OLYMPUS B x 41, Japan) which was equipped with Wild MPS 45 Photo automat Camera Controller.

3. Results and Discussion Characterization of Various Cellulose Fiber Chemical Assay

Qualitative chemical assay was carried out according to the standard procedure. Investigation on the color changes and precipitation, it should be known whether a particular class of aldehyde group was present or not. The results were present in table (1).

Table1. chemical assay of activated cellulose fiber

Reagents	Observation	Remark
2,4-DNP	Red ppt	Aldehyde or Ketone
Tollen	sliver mirror	Aldehyde
Fehling's	Yellow ppt	Aldehyde
Bendict's	Orange ppt	Aldehyde
Schiff's	Magenta color	Aldehyde
Benzidine	Reddish brown	Aldehyde

3.1 Comparison of FT-IR Spectrophotometric Characters between Coconut Cellulose Fibre, Modified Cellulose Fiber and lipase immobilized Cellulose Fiber

Coconut cellulose fiber and modified cellulose fibers can be identified by infrared spectrophotometric analysis. The characteristic bands of coconut white meat (fiber) was observed: $\upsilon_{O\text{-}H}$ at 3380 cm^{-1}, $\upsilon_{C\text{-}H}$ at 2923.9 cm^{-1}, $\upsilon_{c=o}$ at 1654.8 cm^{-1}, aromatic skeletal and $\upsilon_{c=o}$ at 1595 cm^{-1}, aromatic skeletal vibration at 1519.8 cm^{-1}, CH_2 wagging vibration at 1319.2 cm^{-1}, $\upsilon_{c.o\text{-}c}$ at 1153.3 cm^{-1}, $\upsilon_{c\text{-}o}$ at 1033.8 cm^{-1}, $\upsilon_{c\text{-}o}$ in cellulose at 941.2 cm^{-1} and $\delta_{C\text{-}OH}$ in cellulose at 670 cm-1 respectively. In FT-IR spectrum of modified cellulose fiber by IO^{-} the strong band of aldehyde CH = O group was clearly observed at 1724 cm^{-1} Figure (2). Thus the IO^{-} agents could be directly attached to C positions 2 and 3, forming dialdehyde group which were the active site for immobilization of lipase by covalent binding. Furthermore, the FTIR spectrum also confirmed the formation of C=N in immobilized fiber. The vibrational band of aldehyde group (1724 cm^{-1}) become weaker after immobilization and formation of new band of C=N at 1550 cm^{-1} were observed.

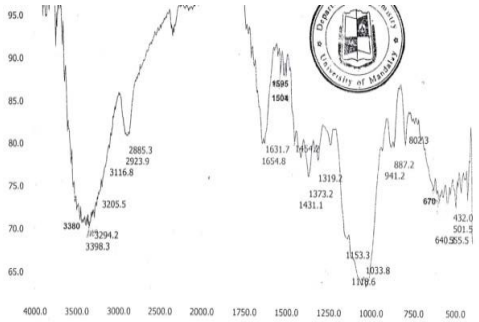


Figure 1. FT IR spectrum of cellulose fiber

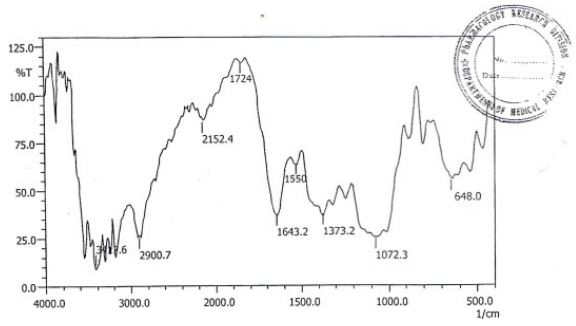


Figure 2. FT IR spectrum of modified cellulose fiber

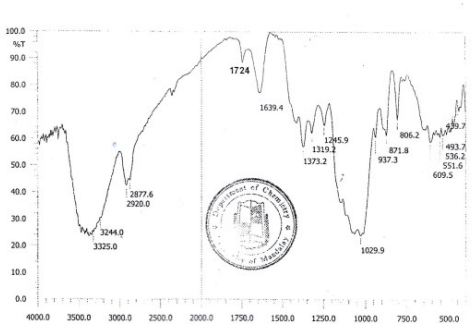
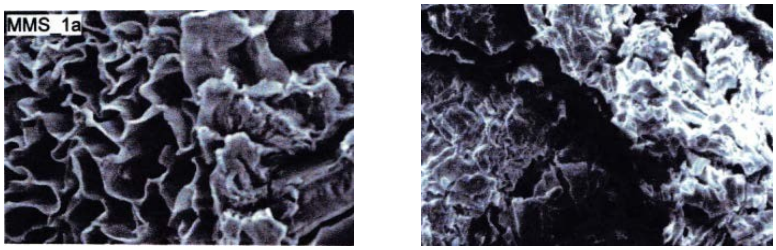


Figure 3. FT IR spectrum of immobilized cellulose fiber

3.2 Determination of Liapse Immobilized Cellulose Fiber by Chemical Assay, Optical Microscopic Images and SEM,

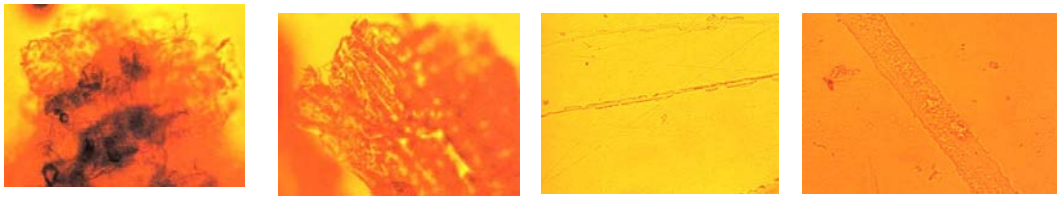
The aldehyde groups of the dialdehyde cellulose were able to react with amino group of lipase to form covalent bonds and resulted in a dialdehyde cellulose immobilized enzyme (Schiff's base formation support- CH = N-ENZYME).The Schiff based bond of immobilized cellouse fiber reacted with $NaNO_2$ and 20% NaOH to give green color and modified cellouse fiber did not react. In SEM analysis , the different texture of fibers were recognized in the micrograph as shown in Figure 4(a and b). Before treatment with $NaIO_4$, the sharp edge with same units were observed at figure (a). After treatment, the deformation of texture was noticed in the SEM due to changes of the chemical treatment figure (b).



(a) (b)

Figure 4.Comparison of SEM of (a) coconut cellulose fiber (b) treated coconut cellulose fiber

The various types of coconut fiber were comparatively studied by Optical microphotographs. The oil droplets were observed in natural coconut endosperm figure 5 (a) but not found in purified coconut fiber figure5 (b). Deviation from the typically indicate alteration of fiber properties by chemical modification (i.e. IO^-) and chemically binding in enzyme immobilization were clearly observed under microscope as shown in photomicrogram (c) and (d). In Figure (c), treated fiber can be seen as rough edge of thread-like tape. In contrast photo image of enzyme immobilized fiber, Figure (d) the cylindrical form was exposed due to the linkage of enzyme irregular form of cross bar at regular intervals along the length of fiber.



(a) (b) (c) (d)

Figure5. Comparison of optical microscopic images of modified cellulose fiber and enzyme immobilized cellulose (a) coconut endosperm (b) defatted cellulose fiber (c) treated fiber and (d) enzyme immobilized fiber

4. Conclusion

Coconut *(Cocos nucifera. L)* has been described as one of Nature's greatest gifts to man because almost every part of the tree is used in some ways or others. Defatted white meat (endosperm) is one of the waste products, very cheap and readily available. With this aspect, the coconut endosperm was chosen as support materials for immobilization of Lipase. The prepared coconut white meat was characterized as cellulose fibre by FTIR spectrum, SEM and Optical-Microscopic images. In order to immobilize lipase enzyme, the cellulose fibre were modified by $NaIO_4$for cleaving vicinal diol groups of glucose units to form dialdehyde as active site. The aldehyde group in modified cellulose fibre were qualitatively confirmed by the positive reaction of chemical assay (2, 4-DNP; Benedict; Tollen; Fheling; Schiffs, and Benzedine) and spectroscopic techniques such as FTIR, SEM and Optical-microscopic images.Positive result of nitrous acid test indicated the presence of 3˚nitrogen immobilized cellulose fiber. Furthermore, the FTIR spectrum also confined the formation of C=N in immobilized fibre. The vibrational band of aldehyde group (1724 cm^{-1}) becomes weaker after immobilization and formation of new band of C=N at 1550 cm^{-1} were observed.

Acknowledgements

The author feel indebted to Professor and Head Dr Ni Ni Than, Department of Chemistry, University of Yangon, Yangon, Myanmar, for his stimulating suggestions.

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Preparation of Microfibrillated cellulose from Bakong (*Hanguana malayana* (Jack) Merr.) and its Application as Paper Reinforcement

Glenn Christian P. Acaso[1] et al

Abstract

One promising source of cellulose is Hanguana malayana (Jack) Merr., commonly known as Bakong, due to its abundance in one region of the Philippines. In this study, microfibrillated cellulose (MFC) was produced from ground Bakong fibers. The fibers were ground using a Wiley mill, pulped using 10% sodium hydroxide, and then bleached with 5% sodium hypochlorite. The process was able to reduce the lignin content from 13.19% to 1.42%. The bleached pulp was then subjected to mechanical grinding using a Masuko Supermasscolloider at 1500 rpm. The overall MFC yield was 41.57% after 15 passes. The MFC was characterized using optical microscopy, scanning electron microscopy and dynamic light scattering. The MFC was then used as an additive to paper sheets produced from Bakong unbleached soda pulp. The burst index, tensile index, tear index, and folding endurance of the 80 g/m^2 handsheets without MFC were 5.98 kPa/m^2-g, 112.09 N-m/g, 6.41 mN-m^2/g, and 621.14 double folds, respectively. The addition of MFC did not significantly affect tensile index and folding endurance. However, burst index decreased with increasing MFC while tear index improved with the addition of MFC.

Keywords: microfibrillated cellulose, paper, non-wood fibers

1. Introduction

Nanocellulose is a highly desirable material due to its abundance, sustainability, and mechanical properties [1]. Upon treatment with mechanical refining equipment, cellulose microfibrils get delaminated. This process is usually accompanied by a significant increase in viscosity of the cellulose suspension after several passes through the equipment [2]. This mechanical disintegration produces microfibrillated cellulose (MFCs), due to the disruption of the intermolecular hydrogen bonding. [1].

Cellulose is very versatile and has been used in pulp and paper industries, textile industries, as building materials, food additives, etc. However, there is an increasing demand for alternative sources of cellulose since the demand for pulp is larger than the wood supply available [3] Several alternative sources of cellulose from biomass and waste products have been studied. [4].

Hanguana malayana, commonly known as Bakong, is a species of perennial herbaceous plant that can be found in Laguna de Cagayan in the town of Santa Teresita, Cagayan. Bakong is a novel and abundant non-wood cellulose source, so soda pulping was used to produce Bakong pulp and microfibrillated cellulose [5, 6].

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2. Methods

Material

Bakong stem fibers were collected from Laguna de Cagayan Handicrafts Association. The fibers had moisture, ash, extractives, and lignin content of 13.12%, 7.09%, 11.03%, and 13.19% respectively. The fibers were cut by hand to lengths of 25 and 50 mm. The 25 mm long fibers were ground using a Wiley mill to lengths less than 2 mm long.

2.1 Pulping of the Bakong fibers

Pressure cooker pulping

200 grams of air-dried 2 mm long Bakong fibers and 20 grams NaOH in 2200 mL distilled water was cooked in a pressure cooker at 120°C for 3 hours. The fibers were then disintegrated using a commercial blender for 1 minute and then bleached by treating with 5% sodium hypochlorite solution at a ratio of 10 grams per 100 mL for 30 minutes.

Digester pulping

600 grams of air-dried 50 mm long Bakong fibers and 120 grams NaOH in 4200 mL distilled water was cooked in a laboratory digester at 170°C for 2.5 hours. The pulp fibers were subjected to disc refining at 0.1 clearance for 2 passes, then beaten using a Valley beater for 5 minutes without load, and another 5 minutes with 5 kg load.

2.2 Production of microfibrillated cellulose (MFC) by friction grinding

Two liters of distilled water was added to 20 grams of bleached pulp sample. The suspension was subjected to a mechanical grinding process using the Masuko Supermasscolloider model MKCA6-2 operated at a speed of 1500 rpm. The suspension was subjected to grinding for 10 and 15 passes, labelled MFC-10 and MFC-15 respectively. Every 1 to 2 passes, the grinder clearance was reduced until a clearance of -10 was reached at the 15th pass. With every pass, the instrument was opened to remove any remaining slurry in the grind stones.

2.3 Incorporation of MFC into Bakong handsheets

The production of the Bakong handsheets was based on the TAPPI Standard T205. 30 grams of digester pulp and MFC at 2%, 6%, and 10% w/w of pulp was diluted in 2000 mL of water and disintegrated using a standard disintegrator at 3000 rpm for 10 minutes. Using a 159 mm diameter TAPPI handsheet former cylinder, 80 g/m^2 handsheets were produced. The handsheets were pressed for 10 minutes at 50 psi and dried at room temperature for 24 hours. The handsheets were then conditioned at an atmosphere with temperature of 23±1°C and 50±2% relative humidity

2.4 Characterization of Samples

Infrared Spectroscopy

The Bakong pulp, bleached Bakong pulp, and Bakong MFC were subjected to Fourier-Transform Infrared Spectroscopy (FTIR) to detect the presence of the characteristic functional groups.

Surface morphology

An optical microscope and a scanning electron microscope were used to capture high-magnification photographs of the surface of the MFC and the Bakong handsheets. Due to their organic and non-conductive nature, the samples were ion-coated prior to viewing under the microscope.

Dynamic Light Scattering

MFC at 0.1% consistency was diluted with HPLC grade water at a concentration of 20% v/v and were subjected to sonication for 10 minutes at ambient temperature. A Malvern Instruments Zetasizer Nano-ZS90 was used to analyze the samples.

Handsheet testing

The grammage of the produced handsheets was determined using ISO standard 534. For testing the mechanical properties, the handsheets were cut according to TAPPI T220. The burst index, tensile index, tear index, and folding endurance of the handsheets were tested according to ISO standards 2758, 1924-2, 1974, and 5626 respectively.

3. Results and Discussion

3.1 Pulping and bleaching of the Bakong fibers

A summary of pulping and bleaching yields is shown in Table 1. The pulping procedure using a pressure cooker was based on a previous study of Mirando [7] performed at the same laboratory. The pulping method resulted in 71.45% yield which is slightly lower than the 77.33% obtained by Jimenez et al. [5]. The lignin content of the resulting Bakong pulp was found to be 8.76%.

Table1. Pulping and bleaching yields, and corresponding lignin content of pulp

Process	Yield (%)	Lignin content (%)
Pressure cooker pulping	71.45 ± 4.89	8.76 ± 1.60
Bleaching of pressure cooker pulp	66.47 ± 3.95	1.42 ± 0.0745
Digester pulping	48.85 ± 3.34	-

The yield of the bleaching method was 66.47% on average. Some of the losses may be attributed to lignin removal. The lignin content of the bleached pulp decreased to 1.42% after the procedure. In addition, the process may have also removed the ash content of the pulp. Some of the losses may be attributed to cellulose oxidation since the reaction pH was not kept above 8. At pH below 8, hypochlorite is in equilibrium with hypochlorous acid, which is considered a powerful carbohydrate oxidant [8].

For digester pulping, pulping yield using the laboratory digester was 48.85% on average. This is much lower than the yield by pulping using the pressure cooker. This may be due to the higher level of alkalinity in the pulping liquor.

3.2 Production and yield of MFC

Table 2 shows a summary of the yields, and consistencies of the MFC produced after 10 (MFC-10) and 15 (MFC-15) passes. With each pass, the grinder was opened and the remaining slurry in the stones was discarded to ensure that all the fibers underwent the same number of passes through the grinder. The yield after producing MFC-10 and MFC-15 was 91.50% and 87.52% respectively. Considering the pulping and bleaching yields, the total yield of MFC production is 43.46% and 41.57% for the MFC-10 and MFC-15 respectively. Increasing the MFC production yield is still very likely, as further improvements in the pulping and bleaching processes may still be done.

Table2. Yields and consistencies of MFC after friction grinding

MFC type	Yield (%)	Consistency (g/L)
MFC-10	91.50	9.23 ± 0.056
MFC-15	87.52	8.86 ± 0.061

3.3 Infrared Spectroscopy

Figure 1 shows the FTIR spectra of the pressure cooker pulp, bleached pulp, and the MFC. The characteristic peaks are compared to the nanocellulose peaks described in the study of Sofla et al.[9]. The samples had broad peaks at the 3200 to 3400 cm^{-1} range, specifying the free O-H groups. At 2896 to 2900 cm^{-1}, there were peaks for C-H stretching vibration. In the 1424 to 1427 cm^{-1} and 1368 to 1370 cm^{-1} region, there were peaks corresponding to C-H bending vibrations, while C-H stretching peaks were present at 2896 to 2900 cm^{-1}. The peaks at 900 to 1100 cm^{-1} corresponds to C-O stretching vibration of the pyranose rings and the alcohol groups.

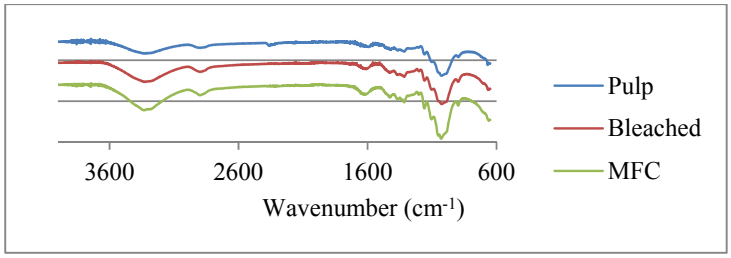

Figure1. FTIR spectra of the pressure cooker pulp, bleached pulp, and the MFC

The functional groups present in the spectra confirm that the pulp, bleached pulp, and microfibrillated cellulose are made up of cellulose. It was observed that there were no significant differences in the FTIR spectra of the three different samples. Thus, based on the peaks present, it can be concluded that the cellulose backbone of the Bakong fibers was not altered significantly during the pulping, bleaching, and grinding process. One peak at around 1600 cm^{-1} corresponded to O-H bending of water, showing that the materials had residual moisture even after drying.

3.4 Morphology of the MFC

Figure 2 shows the optical microscope images, and SEM images of the MFC-10 and MFC-15. It was observed that large fibers 700 µm long and 20 µm wide are still present in the suspension, but much smaller fibers and evidence of microfibrillation were visible. No further treatment was performed to improve the size distribution. The structures surrounding the large fibers suggest that significant defibrillation occurred during grinding. However, the drying method was not able to successfully isolate the individual microfibrils, agglomerating and forming the sheet-like structures.

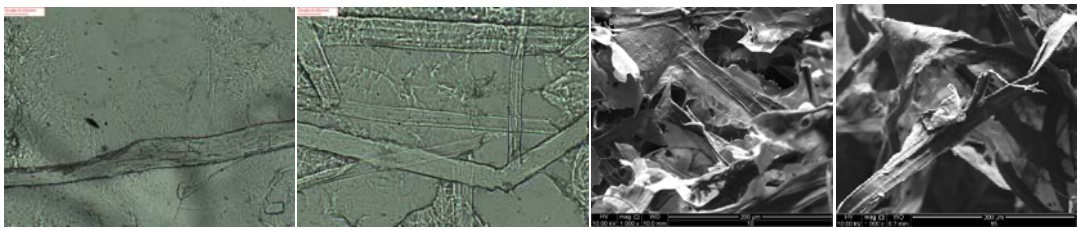

Figure 2. *From left to right: optical microscope images (scale bars: 0.02 mm) of MFC-10 and MFC-15; and SEM images (scale bars: 200 µm) of MFC-10 and MFC-15*

3.5 Size Analysis by Dynamic Light Scattering

Table 3 shows the average particle diameters and polydispersity indices (PDI) of the MFC-10 and MFC-15. Since there were no further treatments to improve the size distribution, the samples had high polydispersity index (PDI) values, and may not be suitable for the DLS technique. However, the technique was only used to observe the difference between the MFC-10 and MFC-15. It was observed that the average size of the suspended MFC-10 was greater than that of the suspended MFC-15, indicating that the increased passes through the grinder further disintegrated the cellulose fibers into smaller particles.

Table 3. Average particle sizes and polydispersity indices

MFC type	Particle size (nm)	Polydispersity Index
MFC-10	857.5 ± 25.55	0.945
MFC-15	537.7 ± 84.57	0.713

The size distribution peaks of the suspended particles are shown in Figure 3. For each type of MFC. The suspended particles from the MFC-10 had two peaks. The peaks for the lower sized particles were at 143.3 nm, 304.2 nm, and 97.57 nm for each of the three trials. While for the higher sized particles, the peaks were at 736.6 nm, 1004.2 nm, and 526.8 nm. For the MFC-15, three peaks were present. The lowest sized peaks were at 345.2 nm, 336.5 nm, and 272.1 nm. The mid-sized peaks were at 1101 nm, 1379 nm, and at 905 nm. The largest sized peaks were at 5255 nm, 5232 nm, and 5160 nm. The relative intensities of the peaks near the 1000 nm region decreased, while the relative intensities of the peaks near the 300 nm region increased as the number of passes of the MFC increased suggesting that the friction grinder was able to reduce the size of the cellulose fibrils.

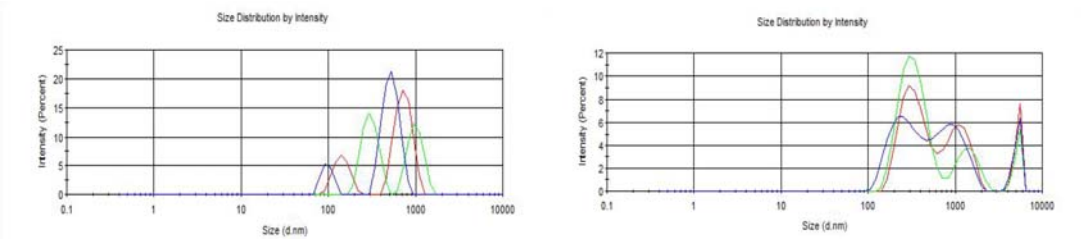

Figure 3. Size distribution of MFC-10 (left) and MFC-15 (right)

3.6 Mechanical Properties of Handsheets with MFC

Figure 4 shows the mechanical properties of 80 g/m^2 handsheets with 0, 2, 6, and 10% MFC-10 and MFC-15 (generated using Design-Expert® Version 11, Stat-Ease, Design Expert V.11.0.3.0 32-bit, Evaluation License, License No. 5611-4228-0954-EVAL, Minnesota, USA).

The burst index of the handsheets decreased with increasing MFC content. This may be due to the MFC not being able to reinforce the inter-fiber bonding in the sheet. The digester pulp was still dark in color so it might have contained a significant amount of lignin which may have not interacted well with the MFC.

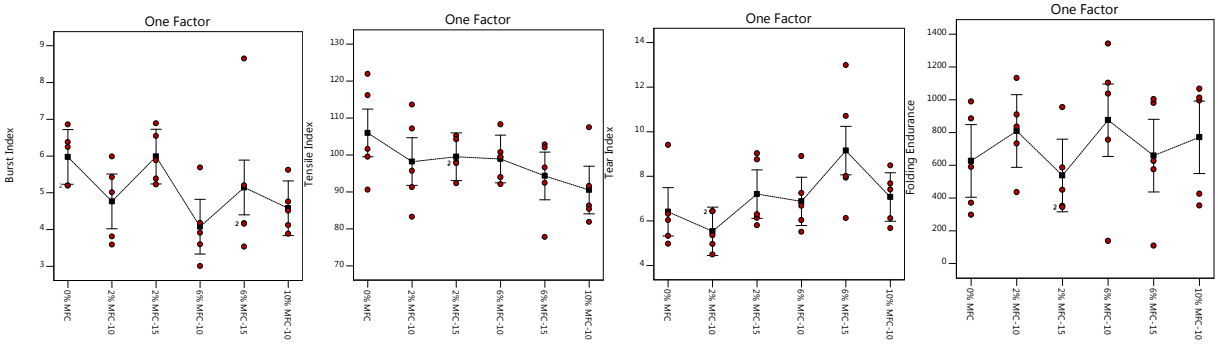

Figure 4. Mechanical properties of 80 g/m^2 paper with MFC

The tensile index of the handsheets did not change significantly with the addition of up to 6% MFC. However, when 10% MFC was added, the tensile index dropped significantly. This further suggests that the inter-fiber bonding was not reinforced; instead, the MFC acted as a filler material that negatively affected bonding. This was like a case in the study of Viana et al. [10], wherein they did not observe any significant improvement in the mechanical property of newsprint with the addition of nanofibrillated cellulose.

As for the tear index, it was observed that the addition of MFC also does not have any significant effect. However, it was observed that the addition of MFC-15 resulted in a better tear index than the addition of MFC-10. This may be due to the MFC-15 being able to occupy the voids between the fibers better than MFC-10, thus adding resistance to tearing.

Lastly, there was no observed improvement in the folding endurance of the handsheets with increased addition of MFC. This may likely be because folding endurance is mostly dependent on fiber length and fiber type, and not on inter-fiber bonding.

4. Conclusions

Handsheets and microfibrillated cellulose were produced from Bakong (*Hanguana malayana* (Jack) Merr.) fibers. Soda pulping was found to be effective in isolating the individual plant fibers. The pulping yields were high, but the pulp still had high lignin content. Bleaching with commercial sodium hypochlorite was effective in further lignin removal. Microfibrillated cellulose (MFC) was produced from bleached Bakong pulp using friction grinding. Grinding yields were 91.50% after 10 passes, and 87.52% after 15 passes. Optical microscopy and scanning electron microscopy supported that microfibrillation occurred, and FTIR analysis showed that the cellulose backbone of the pulp, bleached pulp, and the MFC. Dynamic light scattering analysis was performed and a noticeable decrease in size occurred with increasing passes through the friction grinder. The high yields obtained showed that the method used for producing the microfibrillated cellulose was promising.

The MFC was incorporated at 2, 6, and 10% concentration into the digester pulp slurry during disintegration and it was observed that the burst index of the pulp decreased with increasing MFC loading. However, tear index was slightly improved by incorporating MFC-15 instead of MFC-10. The MFC had no significant effect on the folding endurance and tensile index but at 10% loading, the tensile index dropped significantly, suggesting that the MFC acted more as filler instead of reinforcement. Overall, the Bakong fibers showed promise as a source of pulp for both microcellulose production and papermaking. The MFC yields were relatively high and the handsheets had good mechanical properties.

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Morphological Study and Chemical Composition of Seeds of Avocado Plant

Khin Myo Thwet[1]

Abstract

Avocado plant (*Persea Americana* Mill.) is an important economic tree, belonging to the family Lauraceae. The morphology of this plant was studied and the chemical composition of avocado seed was investigated. *Persea americana* Mill. is an evergreen tree with alternate leaves, panicle cyme with deciduous bracts. Avocado fruit is single seeded berry, which has a large central seed. The chemical results showed that the investigated seeds contain phytochemicals such as alkaloids, flavonoids, tannins, saponin, phenolic compounds, cyanogenic glycoside and steroid. The content of heavy metals were analyzed by using AAS, these results showed higher content of calcium (Ca). The nutritional value from powdered seeds of this plant was revealed the highest content of carbohydrate. According to this research, this avocado seeds contain medicinal and valuable chemical contents.

Keywords: Avocado seed, phytochemical, elemental, nutritional

1. Introduction

The avocado (*Persea Americana* Mill.), a tree with probable origin in South Central Mexico, is classified as a member of the flowering plant family Lauraceae. The fruit of the plant, also called an avocado (or avocado pear or alligator pear), is botanically a large berry containing a single large seed. Avocados are commercially valuable and are cultivated in tropical and Mediterranean climates throughout the world. The species is variable because of selection pressure by humans to produce larger, fleshier fruits with a thinner exocarp.[14] Mexico has the largest production, most of the crop being consumed within the country. Other important producers are: Brazil, United States, Dominican Republic, Indonesia, Peru, Israel and Haiti.[17] This fruit is high calories, contains vitamins A, B and E and is a good source of folic acid, riboflavin, niacin, thiamin, iron and potassium. It is eaten in salads and desserts and used as a sandwich filling and an ingredient in dips and spreads, ice-creams and milkshakes. Kew botanist William Milliken has studied the traditional anti-malarial plants in the state of Roraima in Brazil.[19] The seed is ground and made into an ointment used to treat various skin afflictions, such as scabies, purulent wounds, lesions of the scalp and dandruff. The flesh is also used in traditional medicine. Researches have shown that avocado seeds may improve hypercholesterolemia, and useful in the treatment of hypertension, inflammatory conditions and diabetes. Seeds have also been found to possess insecticidal, fungicidal, and anti-microbial activities. The avocado seed is rich in phenolic compounds, and these may play a role in the putative health effects.[10]

Avocado was introduced into Myanmar over 60 years ago and it was first planted at Pyin U Lwin. The area of avocado was first expanded to the neighbouring towns of the northern Shan State and later to Southern Shan State; also, to Kayah and Chin States. Geographically, all these states are in the hilly regions which have relatively lower temperatures, with favourable annual rainfall. Avocado continues to be cultivated by the farmers as a backyard crop and no commercial plantations exist in the country. The existing scattered trees are classified into three

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categories, which the local people consider as local varieties. These are: a) Anyo Myo, with dark coppery-coloured fruits; b) A Sein Myo, with round green-coloured fruits; and c) Buthi Pon Myo, with oval-shaped and small neck fruits.[3]

In this research, the avocado (buthi pon myo), with oval-shaped and small neck fruit was selected. This fruit is Myanmar namely called htaw bat thi.[5] The seeds of this fruit were investigated for phytochemical tests.

In Myanmar, avocado is not grown for commercial purposes, grown only for consumption within the locality. They are used as fencing trees in home gardening and plantations. This fruit was used as Traditional Burmese avocado salad at Inle Lake, Shan State, Myanmar. Avocados have long been cultivated for their edible fruits, the flesh is highly nutritious and has a composition different from that of other fruits. This plant is important for nutritional and medicinal values. The present research studied the presence of phytochemicals and nutritional values of *Persea Americana* seed.

2. Methods

The specimens of avocado plant used in this research were collected from Taunggyi University Campus, Taunggyi Township, Southern Shan State and studied from May to August, 2019. The fresh parts of this plant were used for morphological characters studies. In morphological study, the fresh plant parts were thoroughly examined and described according to the standard used in Department of Botany, University of Taunggyi.[8] The plants were recorded by taking photographs. The fresh specimens of leaves were washed with water to remove impurities. After washing, the specimens were weighed and air dried at room temperature and weighed intermittently. When constant weight was obtained the samples were completely dried. Then specimens were pulverized by grinding machine and store in air tight containers to prevent moisture changes, contamination and kept for phytochemical screening. Atomic Absorption Spectrometry (AAS) was carried out at University of Research Center, University of Yangon and phytochemical screening was carried out at the Department of Chemistry, Taunggyi University.[12] The nutritional values of this powdered leaves was determined by using Kjeldahl method.

3. Results and Discussion

3.1 Morphological Studies

Family - Lauraceae

Scientific Name - *Persea americana* Mill.

Common Name - Avocado

Myanmar Name - Htaw bat thi

Habit: evergreen tree, up to 22 m tall. Leaves: simple, alternate, margin entire, 10-20 cm long, blade elliptic to lanceolate, leathery above, dark green with prominent midrib. Inflorescence: panicle cyme with hairy flowers, clusters at the ends of branches. Flowers: bisexual, trimerous, greenish yellow; perianth two whorls, 3 outer and 3 inner tepals, 4-5 mm long, tomentose; stamens 9, 3 whorls, 3 staminodes in 1 whorl, 2 orange nectaries at the base of

each inner whorl; carpel with 1 ovary, style slender and stigma capitate. Fruit: fleshy berry, single seed, oval-shaped, 7-15 cm long. Seed: globose, 3.5-4.5 cm in diameter, covered with firm skin. Flowering period: March-June

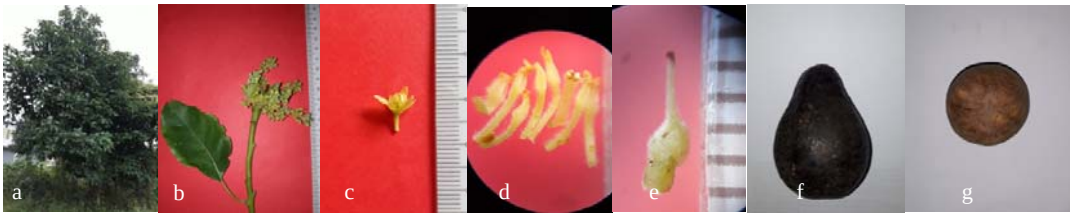

Figure 1 Persea Americana Mill. a) habit, b) inflorescence, c) flower, d) stamens, e) gynoecium, f) fruit, g) seed

3.2 Chemical Studies

Table 1 Preliminary phytochemical screening of powdered seed from Persea americana Mill.

No.	Test	Extract	Test reagents	Observation	Results
1	Alkaloid	1%HCl	Dragendorff's reagent	Orange ppt.	+
2	Amino acid	H_2O	Ninhydrin reagent	Violet color	+
3	Carbohydrate	H_2O	Benedict's solution	Red color	+
4	Flavonoid	EtOH	Mg turning + Conc: HCl	Reddish-pink color	+
5	Glycoside	H_2O	10% Lead acetate	White ppt.	+
6	Phenolic compound	EtOH	10% $FeCl_3$	brown color	+
7	Reducing sugar	H_2O	Benedict's solution	red color	+
8	Saponin	H_2O	Conc: H_2SO_4	Forthiness	+
9	Starch	H_2O	Iodine solution and potassium iodide	deep blue color	+
10	Steroid	EtOH	Acetic anhydride + Conc:H_2SO_4	Green color	–
11	Tannin	EtOH	NaCl, 1% Gelatin	deep blue color	+
12	Terpenoid	EtOH	Acetic anhydride + Conc:H_2SO_4	pink color	+
13	Lipophilic group	H_2O	0.5M KOH	deep color	–
14	Polyphenol	EtOH	1%$FeCl_3$+1%$K_3Fe(CN)_6$	greenish-blue color	+
15	Protein	H_20	NaOH solution, $CuSO_4$ solution	red ppt.	+

(+) = presence, (-) = absence, ppt = precipitate

Phytochemicals are important chemicals which found actually in plant parts. Table 1 show below the present of alkaloid, amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, starch, tannin, terpenoid, saponin, polyphenol and protein in powdered seed of *Persea America* Mill. Some phytochemicals stimulate the immune system and others slow the growth of cancer cells or prevent DNA damage. [19] Flavonoids are a group of bioactive compounds that are extensively found in foodstuffs of plant origin. Various studies in vitro and in vivo showed that flavonoids possess antimicrobial, anticancer, antidiarrheal and radio protective activities.[12] Clinical studies have suggested that saponins affect the immune system in ways that help to protect the human body against cancers and also lower cholesterol levels. Saponins decrease blood lipids, lower cancer risks and lower blood glucose.[23]

Table 2 Nutritional value of powdered seed of Persea Americana Mill

No.	Experiment	Present Chemical Analysis Results
1	Moisture (%)	16.88
2	Ash (%)	0.01
3	Fat (%)	1.75
4	Fiber (%)	4.70
5	Protein (%)	5.40
6	Carbohydrate (%)	71.26

The determination of nutritional values was revealed at table 2. Protein, fiber, fat, ash and moisture, carbohydrate present in the powdered seed of *Persea americana* Mill. was analyzed. Carbohydrate was present higher percent than the others. Carbohydrates are one of the most important food groups in the diet. They provide essential elements that the body needs for instant energy production and various vital functions. Carbohydrate help maintain a healthy blood sugar level.[21] Fiber is important to digestion and regularity, weight management, blood sugar regulation, cholesterol maintenance. According to some scientists, fiber could actually help people live longer.[24]

Table 3 Elemental analysis of powdered seed of Persea Americana Mill

No.	Elements	Mg / L
1	Calcium (Ca)	2.051
2	Manganese (Mn)	0.539
3	Lead (Pb)	-0.764
4	Magnesium (Mg)	1.778
5	Cronium (Cr)	1.063
6	Cadmium (Cd)	0.466
7	Zinc (Zn)	0.22
8	Copper (Cu)	0.141

The elemental analysis of powdered leaves sample showed above table 3 that the presence of high elemental concentration was Calcium (Ca) and the lowest concentration was Lead (Pb). Calcium is very essential in muscle contraction, oocyte activation, building strong bones and teeth, blood clotting, never impulse, transmission, regulating heart beat and fluid balance within cells. Long term of calcium deficiency can lead to oestoporosis in which the bone deteriorates and there is an increased risk of fractures.[9] Exposure to high levels of lead may cause anemia, weakness and kidney and brain damage. Very high lead exposure can cause death.[22]

4. Conclusion

Morphological characters and phytochemical composition of *Persea americana* Mill. have been studied in this research. According to phytochemical investigation, the important chemical composition was found in powdered seed of this plant. Seeds have also been found to possess insecticidal, fungicidal, and anti-microbial activities. The avocado seed is rich in phenolic compounds, and these may play a role in the putative health effects.[10] Thus, further researches concerning with the seed of *Persea americana* will be continuously performed. Also in Myanmar, avocado will be widely grown for more consumption and commercial purposes.

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Ecological Study on Clouded Monitor Lizard *Varanus Nebulosus* (Gray, 1831) In Hlawga Wildlife Park, Yangon Region

Win Zaw[1], Thet Naing (1)[2], Mae May Phaw[3]

Abstract

A total of nine specimens were recorded by means of both captured and visual encountered in the 25 sample plots of Hlawga Wildlife Park from July 2018 to January 2019. Distinctive characters and morphometric data (Weight, Snout-Vent length, Tail length, Head length, and Head width) of the recorded species were given systematically. Population status of *Varanus nebulosus* was analyzed by Quadrat Sampling method, and estimated to the 0.36 individual per plot area (900 m²), 400 individual per square kilometer, and 2496 individuals per study area. The sex ratio of male to female specimen was found to be nearly 1:1. Chi square test (x^2) showed that the sex ratio of the studied specimens was not significantly different (P>0.05). Five types of habitats were categorized in the study area, ground hole (16.1 %), concrete pipe (9.7 %), dump hole (6.5 %), tree-hole (48.4 %), and canopy (19.3 %). The highest frequency of habitat type used was observed for tree hole (TH). Twelve plant species were recorded to be utilized by the species in the study area: the most preferred plants were *Albizia procera* and *Pterospermum acerifoium*. The habitat types of tree-hole and plant canopy were dominantly utilized by the study specimen.

Keywords: Wildlife, population, morphometric data, sex ratio, habitats

1. Introduction

Varanids are an exclusively Old World group of predominantly tropical and subtropical lizards occurring throughout Africa, southern Asia from the Mediterranean coast to southern China, and south to and through most of Australia. Presently, more than 70 species are recognized, and all are contained within the genus *Varanus*. A dozen or so of these monitors have broad distribution, including *Varanus niloticus* throughout much of Africa and *V.salvator* from India and Sri Lanka through Southeast Asia to Sulawesi. All monitors are carnivores, and most are active foragers that search for their prey by sight and smell, having a snake-like long, forked tongue. Varanids are generally solitary and they are almost exclusively diurnal, although some rock- dwelling species may occasionally be crepuscular.

All varanids are oviparous. Captive animals are able to produce two clutches in a year, and wild varanids apparently breed only once a year. Some species (*V.rosenbergi*, *V.varius, V.giganteus, V.albigualris, V.niloticus,* and *V.bengalensis*) lay their eggs in termite mounds which maintain their temperature above 25˚C, with high humidity, throughout winter. Other species simply bury their clutch deep in the soil, while *V.olivaceus* reportedly lays its eggs in hollow trunks and limbs of trees. The age of sexual maturity for most species is most known, but it probably occurs at an older age in large species than in small species. Estimate of *V. komodoensis* takes about five years to mature.

According to [1a], of all the varanids, five species (*V. dumerilii, V. rudicollis, V. salvator, V. bengalensis,* and *V. nebulosus*) are found in Myanmar and Southeast Asia countries. Once, *V. nebulosus* was a subspecies of *V. bengalensis* (*V. bengalensis nebulosus*).

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The present work was conducted on the aim of contributing field data regarding the following **objectives**:

- to assess the population status of *Varanus nebulosus* in Hlawga Wildlife Park,
- to record the morphometric data and sex ratio of the studied species,
- to examine the habitat types and usages of the recorded species, and
- to provide the data for the assessment of National Red List in Myanmar and IUCN Red List.

2. Materials and methods

Hlawga Wildlife Park is situated in Mingaladon Township, Yangon Region and located between Latitude 17° 02′ N to 17 ° 04′ N and Longitude 96 °05′ E to 96 ° 08′ E (Fig. 1). The whole area of the park was about 6.24 kilometer square. The field study was carried out from July 2018 to January 2019. Data collection was undertaken 2-3 days per week with the help of the research members and residential staffs. The specimens were collected alive from the studied habitats by hand capturing, noosing, and visual encountered survey methods. Identification of the studied species was after [2], [3a], [1b] and [4a].

A total of 25 plots (30m × 30m) were randomly set up as sampling plots along both sides of the vehicle road-transect, within 50 m from the road. The distances between plots were not closer than 150 m, and not farther than 1000 m. GPS points were recorded at the centers of all sample plots (Plate. 1). The observation was done in pair of plots with overlaps per a day (for example, No.1 and 2, 2 and 3, 3 and 4, etc.) from 7:00 am to 5:30 pm. The population status for the studied species was investigated and assessed by means of direct observation of the plot pairs. Quadrat sampling method used to estimate the population status of the species.

The body weight (BW), snout-vent length (SVL), tail length (TL), head length (HL), and head width (HW) of the captured specimens from sampling plots were recorded by a digital balance and digital caliper or measuring tape to the nearest gram and millimeter. Unfortunately monitor lizards are notoriously difficult animals for sex differentiation. Sex of monitor lizards is often determined on the basis of hemipenal eversion.

Habitat utilization of the studied species was recorded by their occurrence frequency in different types inhabited by both visual encountered and captured specimens within the whole study area. Five types of habitats were categorized in the study areas as ground hole (GH), concrete pipe (CP), dump hole (DH), tree hole (TH), and canopy (C). Identification of plant species was followed after [5].

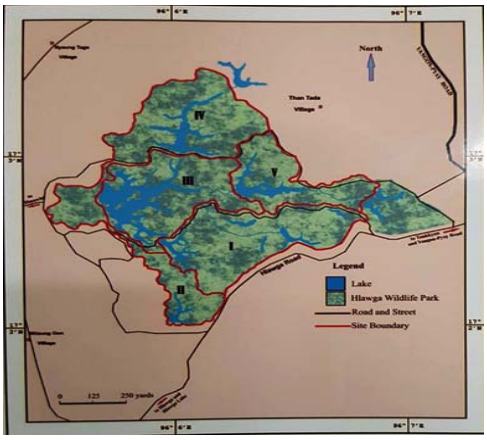

Figure 1. Map of Hlawga Wildlife Park (Source: Topographic map and Google image, 2018)

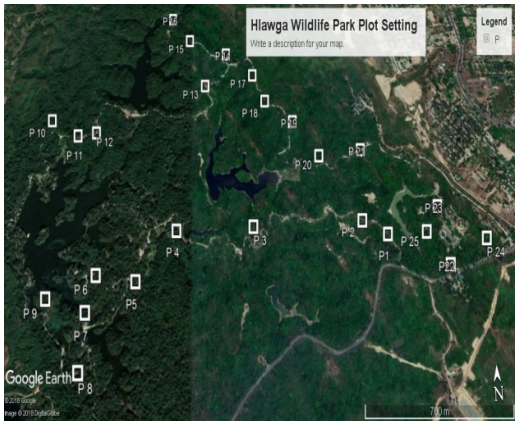

Plate 1. Map showing the plot setting as study sites in Hlawga Wildlife Park (Source: Google Earth Pro. Image © 2019 Maxar Technologies © 2018 Google)

3. Results and Discussion

Population status

In the present study, a total of nine specimens (*Varanus nebulosus*) were observed by means of both captured and visual encountered specimens in the sample plots of Hlawga Wildlife Park. Of the 25 sample plots, the plot numbers 3, 5, 14, 17, 20, 21, and 23 were recorded as captured specimens, and 4 and 22 as visual encountered specimens (VES) respectively. Only one specimen per plot was occurred in these recorded plots. According to the result, the population status of study species was 0.36 individual per plot area (900 m^2), meaning 400 individuals per square kilometer.

The specimen occurrence of the sample plots were plot number 3, 4, 5, 14, 17, 20, 21, 22 and 23. Any specimens were not observed in remaining plots. This was assumed that specimen observed plots were far from human visiting places where monkey population was dense.

Morphometric data of recorded specimens

The morphometric parameters of captured specimens were recorded in this study. The weights and lengths of the collected specimens were recorded to the nearest gram and millimeter.

In this study, the recorded morphometric data of the captured specimens were 451.3±163.41 (232-624) in BW, 273.4 ± 40.4 (230-310) SVL, 416.8 ± 68.2 (340-470.3) TL, 40.4±8.0 (32.2-48.2) HL, 21.2±5.2 (16.3-26.7) HW of males (n=3) and 244.0±36.45 (162-303) BW, 225.4±17.6 (200-240.5) SVL, 367.8±34.0 (320.3-400) TL, 37.2±5.7 (30-43.2) HL, 23.0±2.6 (20.5-24.3) HW of females (n=4) respectively. The 624 g of BW, 310 mm of SVL, 470.3 mm of TL, 48.2 mm of HL, and 26.7 mm of HW were recorded in the largest male specimen; 303 g of BW, 240.5 mm of SVL, 400 mm of TL, 43.2 mm of HL, and 24.3 mm of HW in the largest female *Varanus nebulosus* in Hlawga Wildlife Park. According to the morphometric data, males were found to be generally larger than females in the present study area.

The mean morphometric measurements of adult male *Varanus bengalensis* reported to be about 58 cm in SVL, 150 cm in TL, and 2.7 kg in weight, and of females at 46 cm in SVL, 120 cm in TL, and 1.5 kg in body weight [6]. The mean sizes of adult *V. bengalensis* in southern Sri Lanka were recorded as 50 cm in SVL and 2.5 kg in weight, but about 11kg in weighted specimen had been reported by [7], [8]. In Malaysia, wild *Varanus* species were stated to have morphometric ranges from 20.0 cm to 40.5 cm in SVL and 109 g in body weight [9]. [10a] reported that the mean snout- vent length in *Varanus giganteus* was recorded to be 800 mm, *V. glebopalma* 350 mm, *V. semiremex* 206 mm, *V. glauerti* 230 mm, *V. gilleni* 190 mm, *V. pilbarensis* 170 mm, *V. storri* 130 mm, *V. kingorum* 120 mm, and *V. primordius* 110 mm. [1c] expressed that mean snout to vent length of *Varanus nebulosus* was 1200 mm. In Java, the maximum SVL of male and female *Varanus nebulosus* reached to 750 mm [4b]. [11a] stated that the morphometric ranges of *Varanus bengalensis nebulosus* in Hlawga Wildlife Park, were recorded as body weight (BW) from 400 to 3500 g, snout-vent length (SVL) from 290 mm to 490 mm, tail length (TL) from 320 mm to 790 mm, head length (HL) from 85 mm to 315 mm, and head width (HW) from 18 mm to 37 mm.

As shown in the present results, the maximum morphometric data of weight, snout vent length, head length, and head width were shorter than those recorded by [11b] in the Hlawga Wildlife Park. Besides, the maximum measurement of SVL in the present study was shorter than the mean snout vent lengths of *Varanus* spp. reported by [1d] and [4c].

Sex ratio

Sex differentiation of the captured specimens was followed after [3b]. In the present study, sex ratio of all the captured clouded monitor lizard specimens could not be seasonally analyzed. The sex ratio of male to female specimens in this study was calculated to be 1:1. Chi square test (χ^2) showed that the sex ratios reflecting the number of males to females of the studied species was not significantly different ($p>0.05$) (Table 1). Hence, the sex ratios were recorded for the present is close to unity (i.e., female: male ratio, 1:1).

Table1. Variation in sex ratios for males and females of the recorded species

Male		Female		Chi square value	P-value	Sex ratio
No.	%	No.	%			
3	43	4	57	0.14	0.705	1:1.3

Habitat types and utilization

Habitat utilization of the study species was recorded by occurrence frequency and percentage in each habitat types inhabited by both visual encountered and captured specimens observed within the whole study area. Five types of habitat were recorded in this study as ground hole (GH), concrete pipe (CP), dump hole (DH), tree-hole (TH) and canopy (C). Different habitat types inhabited by the clouded monitor lizard were recorded together with their frequencies and percentages of utilization (Table 2). Occurrence of habitat types utilized by clouded monitor lizard in the studied plots was also recorded. Among the 25 plots, nine were observed and shown in Table 3. According to the data, the tree-hole and canopy were predominant habitats.

[3c] expressed that the clouded monitor lizard had more arboreal than terrestrial habit, unlike Bengal monitor lizard (*Varanus bengalensis*). [11c] investigated that two different types of niche utilization such as arboreal niche on the trees (n= 86, 78.9%) and on the ground (n= 23, 21.1%) were recorded. They were found to prefer tree hollows, or cavities of the trunk of live and dead trees or twigs due to thermo regulation.

Many varanid lizard species are arboreal as adults, particularly the smaller species. Some of the larger species, such as *Varanus tristis*, and *V. varius*, also climb trees, and arboreal habitats are common in some young specimens of the larger species, including *V.komodoensis* ([12]) and *V. varius* ([13]). *Varanus varius*, *V. tristis*, *V. scalaris*, *V. gilleni* and *V. caudolineutus* often use tree holes and loose bark as shelters and *V. prasinus* is aided by its prehesite tail when climbing ([14]). *Varanus glacerti*, *V. glebopalma*, *V. kingorum, V. primordius*, *and V. storri* occur in rocky habitats, although *V. semiremex* inhabits of coastal mangrove forests ([10b]). [15] stated that *Varanus salvator*, water monitor lizard, was observed in sandy and ground habitats in Kyaikhtiyoe Wildlife Sanctuary, Mon State. According to the present study, the tree holes (48.4%), canopy (19.3%), ground hole (16.1%), concrete pipe (9.7%), and dump hole (6.5%) were observed as habitats of the study specimens recorded by means of hand capturing and visual encountering frequencies. Since tree-hole habitat was observed in nine plots, the studied lizard may more prefer than those of other types. The tree-holes inhabited by each studied specimen were two in number and not equal in height. The orientation of tree-holes inhabited by the study species was observed not in uniform. Therefore, it may be assumed that the tree hole used by this species may be the remains of other species.

Table2. Frequencies and percentage of different habitat types utilized by study species

Habitat Types	No. of frequency	Percentage
Ground hole	5	16.1
Concrete pipe	3	9.7
Dump hole	2	6.5
Tree hole	15	48.4
Canopy	6	19.3
Total	31	100.0

Table3. Occurrence of habitat types utilized by clouded monitor lizard in the studied plots

Plot No.	GH	CP	DH	TH	C
Plot 3		√		√	
Plot 4	√			√	
Plot 5				√	
Plot 14				√	√
Plot 17				√	
Plot 20				√	
Plot 21	√		√	√	
Plot 22				√	
Plot 23				√	

Plant species utilized by Varanus nebulosus

Regarding plants used by the study specimen for the tree holes and canopy, a total of twelve plant species were recorded to be inhabited by the during the study period (Table 4). The different frequencies of plant varieties utilized by *V. nebulosus* were shown in Table 5. The highest frequency of use was *Albizia procera* and *Pterospermum acerifolium* as the same frequency of (19.05%), following by *Streblus asper* (14.29%) at the second highest frequency. Tree-hole was the highest utilized (15 and 48.4%) part of the trees. In the habitat utilization, the tree species of *Albizia procera* and *Pterospermum acerifolium* were the most utilized by the clouded monitor lizard, *Streblus asper* was the second most used tree species in the Hlawga Wildlife Park. This may be due to easy penetration of these plants than those of other plant species.

Table4. Plant species utilized by the clouded monitor lizard in Hlawga Wildlife Park

Sr.No.	Scientific Name	Myanmar Name	Family	Habitat
1.	*Melanorrohoea usitata* Wall.	Thitsi	Anacardiaceae	Tree
2.	*Alstonia scholaris* (L.) R.Br	Taung-mayo	Apocynaceae	Tree
3.	*Markhamia 54tipulate* (Wall.) Seem. exK. Schum.	Ma-hlwa	Bignoniaceae	Tree
4.	*Elaeocarpus robustus* Roxb.	Taw-magyi	Elaeocarpaceae	Tree
5.	*Albizia procera* (Roxb.)	Sit	Fabaceae	Tree
6.	*Senna siamea* Lam.	Mezali	Fabaceae	Tree
7.	*Samanea saman* (Jacq.) Merr.	Kokko	Fabaceae	Tree
8.	*Butea monosperma* (Lam.) Taub.	Pauk	Fabaceae	Tree
9.	*Tectona grandis* L.F	Kyun	Limaceae	Tree
10.	*Pterospermum acerifolium* (L.) Willd	Nagye	Malvaceae	Tree
11.	*Streblus asper* Lour	Okhne	Moraceac	Small Tree
12.	*Anthocephalus morirndaefolius* Korth.	Ma-u	Rubiaceae	Tree

Table 5. Frequencies and percentage of plant species utilized by the study species

Sr.No.	Plant species	Tree hole	Canopy	Total	(%)
1	*Melanorrohoea usitata*	1	1	2	9.52
2	*Alstonia scholaris*	1	0	1	4.76
3	*Markhamia stipulate*	1	0	1	4.76
4	*Elaeocarpus robustus*	1	0	1	4.76
5	*Albizia procera*	2	2	4	19.05
6	*Senna siamea*	0	1	1	4.76
7	*Samanea saman*	1	0	1	4.76
8	*Butea monosperma*	1	0	1	4.76
9	*Tectona grandis*	1	0	1	4.76
10	*Pterospermum acerifolium*	3	1	4	19.05
11	*Streblus asper*	2	1	3	14.30
12	*Anthocephalus morirndaefolius*	1	0	1	4.76
Total		15	6	21	100.00

4. Conclusions

In this study, a total of nine specimens of the clouded monitor lizard *Varanus nebulosus* were recorded from 25 sample plots (22500 square meters). According to the analyzed data, the population density of the studied species was estimated as 0.36 individual per plot area (900 square meters), and 400 individuals per square kilometer. According to the present result, the population status of the studied species was estimated to be 2496 individuals inhabiting in the study area (6.24 kilometer square).

Because the recorded maximum morphometries (BW, SVL, TL, HL, and HW) of the largest specimen in the present study were shorter than that of the specimens recorded by [11d], and the maximum measurement of SVL was shorter than the mean snout vent lengths of *Varanus* spp. reported by [1e] and [4d]. Thus all the captured specimens of *Varanus nebulosus* in this study were concluded to be juveniles and sub-adults. Because the study period covered only the raining season and the early cold season, the adult *Varanus nebulosus* could not be caught in this study.

The Chi square test ($p>0.05$) of the sex ratio 1:1 revealed the captured number of male to female specimens in this study were not significantly different and close to unity.

Although five habitat types were utilized by the clouded monitor lizards, the most preferred habitat type was the tree-hole in twelve tree species of the study site.

The present work hoped to impart some information on the population status and habitat utilization of *Varanus nebulosus* which could partially contribute to data base for conservation of the wildlife.

Acknowledgements

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Investigation of Phytochemicals and Some Bioactivities of *Smallanthus sonchifolius* (Yacon) Leaves

Khin Chaw Win[1], Ni Ni Than[2], Hla Ngwe[3]

Abstract

This research aims to investigate phytochemical constituents and some biological activities from the leaves of Smallanthus sonchifolius (Yacon). The sample was collected from Ywar-ngan Township, Southern Shan State. The preliminary phytochemical tests revealed the presence of alkaloids, □-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids in Yacon leaves, however, cyanogenic glycosides and starch were found to be absent. Nutritional values such as moisture (9.19 %), fat (3.08 %), ash (15.96 %), fibre (11.75 %), protein (11.77 %), carbohydrate (48.25 %) and energy value (247 kcal/100g) of leaves were determined by AOAC methods. Antimicrobial activity of pet-ether, ethyl acetate and ethanol extracts of leaves of Yacon inhibited all species of microorganisms such as B. subtilis, S. aureus, P. aeruginosa, B. pumilus, C. albicansand E.coli with the inhibition zone diameters in the range between13~25 mm. The watery extract (IC50 = 4.54 □g/mL) was found to be the slightly more potent than ethanol extract (IC50 = 5.71 □g/mL) in antioxidant activity. The 50 % α- amylase inhibition concentrations (IC50) of water and ethanol extracts were respectively to be 290.60 and342.90 μg/mL indicating these two extracts possessed α-amylase inhibition activity.

Keywords: nutritional values, antioxidant, antimicrobial, α-amylase inhibition activity

1. Introduction

Yacon is a plant, a species of the family Asteraceae (Compositae), with the botanical name *Smallanthus sonchifolius.* The aerial part is formed by large leaves and a flower with yellow, radially arranged petals [1]. Antidiabetic medicinal properties were attributed mainly to Yacon leaves. Yacon water extracts induced an increase in the plasma insulin concentration [2]. It is cultivated up to a height of 3, 200 m above sea level. Yacon cultivation has been expanded to several countries such as Japan, New Zealand, Czech Republic, Brazil [3]. In Myanmar, Yacon was cultivated in Ywar-ngan Township, Southern Shan State. In the research was aimed to study the biological investigation including antioxidant activity, antimicrobial activity, α-amylase activity and chemical investigation such as phytochemical tests and nutritional values, of leaves of *Smallanthus sonchifolius* (Yacon)

2. Methods

Sample Collection and Preparation

In this research, leaves of *S. sonchifolius* (Yacon) were collected from Ywar-ngan Township, Southern Shan State. Then, the sample was identified at the Department of Botany, University of Yangon. The collected samples were cleaned by washing them with water and air-dried at room temperature for two weeks. The air- dried samples were ground into power by means of a grinding mill and stored separately in air-tight containers to prevent the moisture and

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other contaminations. The dried powdered sample was used to investigate for their chemical and biological activities

Preliminary Phytochemical Tests

A few grams of each sample were subjected to the tests of alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, flavonoids, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins and terpenoids as the preliminary phytochemical test according to reported methods.

Nutritional values

Nutritional values such as moisture content, ash content, fat content, fibre content, protein content, carbohydrate content and energy value of the selected sample were determined by AOAC method [4].

Antimicrobial activity

The antimicrobial activity of five crude extracts as petroleum ether, chloroform, ethyl acetate, ethanol and water from the leaves of Yacon was determined against six strains of microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli* by employing agar well diffusion method [5].

Procedure for antioxidant activity

The free radical scavenging activity of crude extract of leaves of Yacon was measured using DPPH free radical scavenging assay [6]. The control solution was prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of ethanol in the brown bottle. The sample solution was also prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of test sample solution. These bottles were incubated at room temperature and were shaken on shaker for 30 minutes. After 30 minutes, the absorbance of different concentrations (1.25 µg/mL, 2.5 µg/mL, 5 µg/mL, 10 µg/mL and 20 µg/mL) of tested sample was measured at 517 nm using UV-7504 spectrophotometer. Absorbance measurements were done in three times for each concentration and the mean value so obtained were used to calculate percentage of radical scavenging activity (% RSA) by the following equation. $\% RSA = [A_{DPPH} - (A_{Sample} - A_{Blank}) / A_{DPPH}] \times 100$

Where, % RSA = % radical scavenging activity of test sample A_{DPPH} = absorbance of DPPH in EtOH solution A_{Sample} = absorbance of sample+ DPPH solution

A_{Blank} = absorbance of sample + EtOH solution

The antioxidant power (IC_{50}) is expressed as the test substances concentration (µg/mL) that result in a 50 % reduction of initial absorbance of DPPH solution and that allows to determine the concentration. IC_{50} (50 % inhibition concentration) values were calculated by linear regressive excel program.

In *Vitro* α-Amylase Inhibitory Assay

In *Vitro* α-amylase inhibitory assay, 3,5- dinitrosalic acid (DNS) method was used to estimate α-amylase inhibition activity, by the reducing sugar (maltose) liberated under the assay conditions. The enzyme inhibition activity was described as a decrease in units of maltose liberated [7].

3. Results and Discussion Preliminary Phytochemical Investigation

From the preliminary phytochemical investigation, it was found that alkaloids, α-amino acids, carbohydrate, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids were present in leaves of *S. sonchifolius*, however, cyanogenic glycosides and starch were found to be absent.

Nutritional Value

The nutritional values: 9.19 % of moisture, 15.96 % of ash, 3.08 % of fat, 11.75 % of fibre, 11.77 % of protein, 48.25 % of carbohydrate and 247 kcal/100g of energy value were determined in leaves of Yacon. The leaves of Yacon are rich sources of protein and carbohydrates.

Antimicrobial Activity

Antimicrobial activity was determined by agar well diffusion method. PE, EtOAc and EtOH extracts of leaves of *S. sonchifolius* inhibited all species of microorganisms such as *B. subtilis*, *S. aureus*, *P. aeruginosa*, *B. pumilus*, *C. albicans*and *E.coli* with the inhibition zone diameters in the range between 13~25 mm. However, H_2O extract exhibited five species of microorganisms except *C. albicans*with the inhibition zone diameters in the range between 11~13 mm. From this investigation, it could be deduced that leaves of Yacon may be effective for the treatment of some diseases such as skin rashes, kidney failure or neurologic problems and diarrhea infected by the test microorganisms. These results are showed in Table 1.

Table 1 Antimicrobial Activity of Leaves of Yacon against Six Microorganisms

Organisms	Inhibition zone diameter of extracts(mm)			
	Pet-ether	Ethyl acetate	Ethanol	water
Bacillus subtilis	19	14	18	12
Staphylococcus aureus	19	15	17	11
Pseudomonaus aeruginosa	25	16	18	12
Bacillus pumilus	20	18	15	11
Candida albicans	15	15	13	-
Echerichia coli	20	15	23	13

Diameter of agar well=10mm, 10mm ~ 14mm = resistant, 15mm ~ 19mm =intermediate, 20mm above = susceptible, No activity = (-)

Antioxidant Activity

The antioxidant activity was studied on 95 % ethanol and water extracts of leaves of Yangon by using DPPH assay according to the spectrophotometric method. In this experiment, five different concentrations (1.25µg/mL, 2.5µg/mL, 5µg/mL, 10µg/mL and 20µg/mL) of each crude extract in 95 % ethanol solvent were used. Ascorbic acid was used as standard and ethanol without crude extract was employed as control. From these experimental results, IC_{50} values were found to be 5 .71 µg/mL for 95 % ethanol extract and 4.54 µg/mL for water extract. However, it was observed that these two extracts have the lower antioxidant activity than standard ascorbic acid (IC_{50} = 1.92 µg/mL). The water extract (IC_{50} = 4.54 µg/mL) is higher potency than ethanol extract (IC_{50} = 5.71 µg/mL) in the antioxidant activity. These results are showed in Table 2 and Figure 1.

Table2. Radical Scavenging Activity (% RSA) and IC_{50} Values of Crude Extracts from Leaves of Yacon and Standard Ascorbic Acid

Extracts	% RSA±SD of different concentrations (µg/ml)					IC (µg/mL)
	1.25	2.5	5	10	20	
Water	37.96 ± 0.49	42.43 ± 1.62	51.72 ±2.76	63.42 ±1.78	76.15 ± 3.57	4.54
Ethanol	39.98 ± 2.02	43.99 ± 2.83	48.38 ± 0.13	59.73 ± 2.43	72.33 ± 1.62	5.71
Standard Ascorbic Acid	45.58 ± 2.88	55.53 ± 1.13	64.82 ± 3.59	73.32 ± 5.78	75.23 ± 6.81	1.92

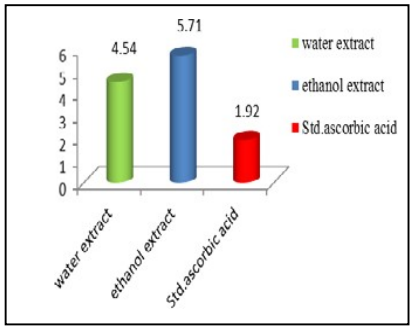

Figure 1. IC_{50} value of water and ethanol extracts from leaves of Yacon and

α-Amylase Inhibition Activity

Hyperglycemia has been a classical risk in the development of diabetes and the complications associated with diabetes. Therefore control of blood glucose levels in critical in the early treatment of diabetes mellitus and reduction of macro and microvascular complications. One therapeutic approach is the prevention of carbohydrate absorption after food intake, which is facilitated by inhibition of enteric enzymes including α- glucosidase and α-amylase present in the brush borders of intestine. In this study, the α-amylase inhibitory activity of leaves of Yacon was investigated. The inhibitory effect of ethanol extract and water extract were analyzed. The percentage inhibition of α-amylase by ethanol and water extracts were studied in a concentration range of 25-400 µg/mL. The percentage inhibition of the samples on α-amylase enzyme activity increased with increasing the concentrations. The ethanol and water extracts of leaves of Yacon was also explored for the in *vitro* α-amylase inhibition and their activity was compared with standard antidiabetic drug, acarbose. The IC_{50} values of ethanol and water extracts of leaves of Yacon were observed to be 342.90 and 290.60 µg/mL. These two extracts possessed α-amylase inhibition activity. However, these two extracts exhibited lower potent α-amylase inhibition

activity than acarbose (IC_{50}=168.02 µg/mL). These observations are dedicated with a bar graph diagram in Figures 2 and Table 3**.** This results suggested that crude extracts inhibited blood glucose elevation by inhibiting α-amylase activity.

Table 3. Amylase Inhibition Activity of Ethanol and Water Extracts of Yacon Leaves and Standard Acarbose

Sample	% Inhibition (mean ± SD) in different concentrations (µg/mL)					IC_{50} (µg/mL)
	25	50	100	200	400	
Watery	19.73 ± 2.91	23.40 ± 1.89	29.39 ± 3.38	42.59 ± 0.96	58.97 ± 1.09	290.60
ethanol	24.39 ± 1.74	31.67 ± 1.89	37.79 ± 1.05	43.36 ± 0.65	52.66 ± 0.53	342.90
Std. Acarbose	15.72 ± 2.18	29.18 ± 2.77	39.78 ± 1.33	54.80 ± 0.88	60.34 ± 0.39	168.02

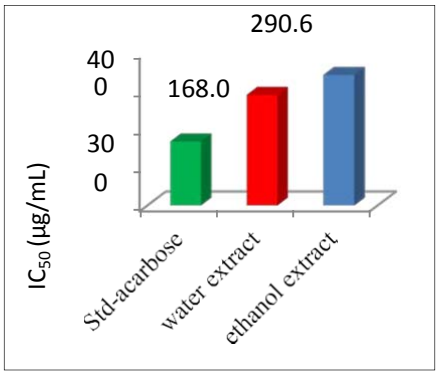

Figure 2. IC_{50} value of water and ethanol extracts from leaves of Yacon and standard acarbose

4. Conclusions

From these observations, Yacon leaves exhibited promising antimicrobial, antioxidant and *in vitro* α-amylase inhibition activities. Yacon leaves were generally found to possess α–amylase inhibitory effect and they may be useful for the formulation of α– amylase inhibitors to manage the Type 2 diabetes mellitus. Therefore, this research work is hoped to contribute to the development of Myanmar traditional medicinal formulation using plant sources for the treatment of Type 2 diabetes, non-insulin dependent diabetes.

Acknowledgements

The authors would like to thank the Department of Higher Education, Ministry of Education, Yangon, Myanmar, for the permission of doing this research.

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Investigation of Chemical Constituents and Bioactivities of Some Organic Compounds from Rhizomes of *Boesenbergia pandurata* (Roxb.) Schltr. (Seik-Phoo) (Red and Yellow Varieties)

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Abstract

The present work deals with the screening of antimicrobial activity and antioxidant activity of *B. pandurata* (red and yellow) rhizomes. Moreover, isolation of some bioactive compounds was obtained from B. *pandurata* (yellow) rhizome. These compounds were characterized by colour reactions, UV and FT-IR, 1D and 2D NMR. These compounds may be deduced as pinostrobin (5-Hydroxy-7-methoxy Flavanone), panduratin A, pinocembrin (5,7-dihydroxy Flavonone), 2', 6' dihydroxy-4'-methoxy chalcone, cardamonin (4', 6' dihydroxy-2'-methoxy chalcone), alpinetin (7-hydroxy , 5-methoxy flavanone) and helichrysetin (4,4',6' trihydroxy-2'methoxy chalcone) respectively. Antimicrobial activity of four extracts (PE, EtOAc, EtOH and H_2O) of both *B. pandurata* (red and yellow) rhizomes was studied on 12 strains of microorganisms by agar disc diffusion method. In antioxidant activity, EtOH and EtOAc extracts of both plants and isolated compounds from *B. pandurata* (yellow) rhizome were measured the antioxidant activity by DPPH staining method.

Keywords: Boeseribergia *panadurata* (Roxb.)[1], Antimicrobial activity [2], Antioxidant activity [3]

1. Introduction

Boesenbergia species is a member of Zingiberaceae. It is composed of approximately 80 species worldwide and 27 species of the genus from Indochina. *Boesenbergia pandurata* (Roxb.) Schltr. (Seik-Phoo) belongs to the Zingiberaceae family. It is one of the ginger plants that are found in South East Asia. This ginger found from some tropical countries including Indonesia, Malaysia, Thailand and Myanmar (Ching et al., 2007). Flavonoids are large secondary metabolites found in rhizome of *B. pandurata*. More than 51 flavonoid compounds from *B. pandurata* rhizome have been isolated. Three classes of main flavonoids are chalcones, flavanones and flavones classified according to their skeletons. The rhizomes are given as tonics and skin liniment. It has been widely used as a medicinal plant, was reported to possess significant anti-oxidative properties. This rhizomes contain essential oil component such as metylcinnamate, champhor, cineole. *B.pandurata* rhizomes also contain saponin and flavonoid such as pinostrobin, pinocembrin, alpinetin, cardamonin, and panduratin (Ching et al. 2007). Phenolic compounds such as flavonoid which contain hydroxyls, are responsible for the radical scavenging effect in the plants. *B. pandurata* rhizomes is used to treat some diseases such as stomach disorders, reproductive infections, kidney stones, or laxative. It has been widely used as a medicinal plant, was reported to possess significant anti-oxidative properties (Chahyadi, 2014).

2. Material and Methods

Collection of Plant Samples

The rhizome of *B. pandurata* (red and yellow) was collected from Yangon and Bago division from October to March. The collected sample was identified by authorized botanists, in

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Department of Botany, University of Yangon. The cleaned and air dried rhizome was made into powered and stored in air tight container.

Figure1. Boesenbergia pandurata (Roxb.) Schltr. (a) yellow plant (b) red plant

Screening of Antimicrobial Activity on Effects of Crude Extracts

Crude extract samples were investigated by were determined by 12 strains of which include *Proteus morganii*, three species of *E. coli*, two species of *Salmonella*, two species of *Vibrio* , two species of *Staphylococcus*, *Shigella dysenteriae* and *Candida albican* by agar disc diffusion method (R. Cruickshank,1975).

Isolation of some Organic Constituents from *B. pandurata*

The dried powder sample of rhizome of *B. pandurata* (500g) was firstly extracted by maceration with 70 % ethanol for two weeks. This ethanol extract was filtered and then concentrated under reduced pressure at 45 ºC using rotatory evaporator. A chromatography was carried out by elution with the solvent system petroleum ether and ethyl acetate ratios of 9:1 to increasing polarity. *B. pandurata* rhizome have proved to be a rich source of flavanones and chalcones including pinostrobin (5-Hydroxy-7-methoxy Flavanone), panduratin A, pinocembrin (5,7-dihydroxy Flavonone), 2', 6' dihydroxy-4'-methoxy chalcone, cardamonin (4', 6' dihydroxy-2'-methoxy chalcone), alpinetin (7-hydroxy,5-methoxy flavanone) and helichrysetin (4,4',6' trihydroxy-2'methoxy chalcone) respectively .

Rapid Screening of Antioxidant by Dot-Blot and DPPH Staining

3 mg of plant extract was dissolved with 6 ml of suitable solvent and was carefully loaded on a 6 cm x 6 cm TLC layer (Silica gel 60 F_{254}: Merck) and dried for 3min. Drops were loaded in order of increasing amount along the plate, 50-400 µg. The sheet bearing the dry spots was placed upside down for 10 seconds in a 0.4 mM DPPH solution. Then the excess solution was removed with a tissue paper and the layer was dried with a hair-dryer blowing cold air. The stained silica layer revealed a purple background with yellow spots at the location of the drops, which showed radical scavenger capacity. The intensity of the while color depends upon the amount and nature of radical scavenger present in the sample (Heng-Yuan Chang, 2007).

3. Results and Discussion

Screening of Antibacterial Activity on Effects of Crude Extracts

Water, petroleum-ether, ethyl acetate and ethanol extracts (red and yellow rhizome) have been tested against *E.coli* species, *Salmonella* species, *Staphylococcus species*, *Vibrio species*, *Shigella dysenteriae*, *Candida albican* and *Proteus morganii* (zone of inhibition ranged from 14 to 23 mm). Water extract did not show any significant activity on all test organisms.

Identification of Some Phytoconstituents

pinostrobin (compound-1) was colourless crystal from ethanol extract**.** uv-vis (MeOH and MeOH/NaOH, λ_{max}/nm) 288, 325 (sh) and 288, 357 (sh); ($AlCl_3$ and $AlCl_3$/HCl, λmax) 293, 324 (sh) and 307, 385 (sh); (NaOAc and NaOAc/H_3BO_3) 288, 325 (sh). FT-IR (KBr, cm^{-1}): 3459 (ν_{OH}), 2973, 2939 (ν_{CH}), 1651 ($\nu_{C=O}$), 1583, 1444 ($\nu_{C=C}$), 1383, 1340 (($\delta_{ip\text{-}CH3}$), 1210, 1159 ($\nu_{as\ C\text{-}O\text{-}C}$), 1092 ($\nu_{s\ C\text{-}O\text{-}C}$).^{1}H NMR ($CDCl_3$, 300 MHz, δ/ppm) : 7.3-7.6 (5H, m), 6.1 (2H, d, J=2.72 Hz), 5.4 (1H, dd, J=12.84 Hz, 2.76 Hz), 3.8 (s, OCH_3), 3.06 (1h, dd, J=15.14/12.84 Hz), 2.9 (1H, dd, J=14.68/2.76 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz, δppm): 79.22 (C-2), 43.34 (C-3), 195.8 (C-4), 164.2 (C-5), 95.15 (C-6), 167.9 (C-7), 94.27 (C-8), 162.8 (C-9), 103.2 (C-10), 138.4 (C-1), 126.1 (C-2', C-6'), 128.9 (C-3', C-4', C-5'), 55.68 (OCH_3); in HMBC ($CDCl_3$, δppm) correlation assignments of H-3 (cis) and H-3 (trans) at δ 3.06, 2.9 and C-2 at 79.22, C-4 at 195.8, C-1' at 138.4, H-6 and H-8 at δ 6.1 and C-5 at 164.2, C-6 at 95.15, C-7 at 167.9, C-8 at 94.27, C-9 at 162.8, C-10 at 103.2; ^{1}H-^{1}H COSY ($CDCl_3$) the correlation between H-3 and H-2 was observed; in HMQC ($CDCl_3$, δppm) correlation assignments of H-2, H-3 (Cis) H-3 (trans), H-6, H-7, 7-OCH_3 at δ 5.4, 2.9, 3.06, 6.08, 6.1, 3.8 and C-2 at 79.22, C-3 at 43.34, C-6 at 95.15, C-8 at 94.27, O-CH_3 at 55.68 were observed.

panduratin A (compound-2) was obtained from ethanol extract and recrystallized with methanol to give colorless solid. uv-vis (MeOH and MeOH/NaOH, λmax/nm) 229. 290; ($AlCl_3$ and $AlCl_3$/HCl, λmax) 322; FT-IR (KBr, cm^{-1}): 3444 (ν_{OH}), 2937 (ν_{CH}), 1631 ($\nu_{C=O}$), 1463 ($\nu_{C=C}$), 1383, 1342 (($\delta_{ip\text{-}CH3}$), 1210, 1180 ($\nu_{as\ C\text{-}O\text{-}C}$), 1109, 1029 ($\nu_{sy\ C\text{-}O\text{-}C}$), 933 ($\delta_{oop\text{-}CH}$). ^{1}H NMR (400 MHz, CD_3OD, δppm), 5.96 (H-3, br, s), 5.79 (H-5, br, s), 3.71 (4-OMe, S), 4.52 (H-1', m), 2.52 (H-2', m), 1.78 (3'-Me, s), 5.43 9H-4', m), 2.32 (H5'(a), m), 2.07 (H-5'(b), m), 3.37 (H-6', br ddd, J=4.6/11.6/11.6 Hz),2.32 (H-1", m), 2.24 (H-1"(b), m), 4.89 (H-2", m), 1.50 (3"-Me, s), 1.50 (H-4",s), 7.02-7.19 (m, H-4"', 5"', 6"', 2"', 3"'). ^{13}C NMR (CD_3OD, 400 MHz, δppm): 106.1 (C-1), 168.5 (C-2), 97.2 (C-3), 165.9 (C-4), 56.2 (4-Ome), 92.2 (C-5), 164.4 (C-6), 55.5 (C-1'), 44.1 (C-2'), 138.4 (C-3'), 23.2 (C-3'-Me), 122.1 (C-4'), 37.2 (C-5'), 38.6 (C-6'), 29.9 (C-1"), 125.7 (C-2"), 132.5 (C-3"), 18.1 (3"-Me), 25.9 (C-4"), 149.5 (C-1"'), 128.2 (C-2"', C-6"'), 125.6 (C-4"'), 129.2 (C-3"', C-5"'), 207.4 (C=O) were observed.

pinocembrin (compound-3) was yellow needle-shaped crystal from ethanol extract. uv-vis (MeOH and MeOH/NaOH, λ_{max}/nm) 290, 325 (sh) and 246, 323 (sh), ($AlCl_3$ and $AlCl_3$/HCl, λ_{max}/nm) 307, 373 (sh), (NaOAc and NaOAc/H_3BO_3, λ_{max}/nm) 253 (sh), 324 and 291, 325; FT-IR (KBr, cm^{-1}) : 3450 (ν_{OH}), 2973, 2939 (ν_{CH}), 1634 ($\nu_{C=O}$), 1616, 1580, 1480, 1435 ($\nu_{C=C}$), 1361, 1302 (($\delta_{ip\text{-}CH3}$), 1212, 1181 ($\nu_{as\ C\text{-}O\text{-}C}$), 1109, 1092 ($\nu_{sy\ C\text{-}O\text{-}C}$), 837, 769 ($\delta_{oop\text{-}CH}$), ^{1}H NMR (300 MHz, CD_3OD, δppm): 7.3-7.6 (5H, m), 5.9 (2H, H-8, H-6, d, J=1.84 Hz), 5.4 (1H, H-2, dd, J=12.84/3.68 Hz), 3.1 (1H, H-3, dd, J=17.4/12.88 Hz), 2.7 (1H, H-3, dd, J=16.96/3.68 Hz); ^{13}C NMR (CD_3OD, 125 MHz, δppm) : 80.44 (C-2), 44.19 (C-3), 197.31 (C=O), 165.49 (C-5), 96.23

(C-6), 168.44 (C-7), 97.18 (C-8), 164.62 (C-9), 103.38(C-10), 140.43 (C-1'), 129.62 (C-3', C-5'), 129.70 (C-4'), 127.34 (C-2', C-6'); in HMQC (CD_3OD, δppm), C-6 and C-8, correlated to H-8 and H-6, C-2 and C-3 correlated to H-2 and H-3 (cis), H-3 (trans), ^1H-^1H COSY (CD_3OD) the correlation between H-2 and H-3 was observed. 2', 6'-dihydroxy, 4'-methoxy chalcone (compound-4) was pale yellow needle-shaped crystal from ethanol extract. uv-vis (MeOH and MeOH/NaOH λ_{max}/nm) 288 (sh), 344 and 290 (sh), 383, ($AlCl_3$ and $AlCl_3$/HCl, λ_{max}/nm) 291 (sh), 345 and 291 (sh), 386, (NaOAc and NaOAc/H_3BO_3, λ_{max}/nm) 291 (sh), 336. This data are identical with the reported data (Markham and Jaipetch, 1982); FT-IR (KBr, cm^{-1}) : 3447 (ν_{OH}), 2995 (ν_{CH}), 1620 ($\nu_{C=O}$), 1589, 1490, 1435 ($\nu_{C=C}$), 1361, 1305 (($\delta_{ip\text{-}OCH3}$), 1212, 1180 ($\nu_{as\ C\text{-}O\text{-}C}$), 1110, 1072, 1056 ($\nu_{sy\ C\text{-}O\text{-}C}$), 987, 956, 895 ($\delta_{oop\text{-}CH}$) were observed.

cardamonin(compound-5) was yellow needle-shaped crystal from ethanol extract. uv-vis (MeOH and MeOH/NaOH, λ_{max}/nm) 288 (sh), 342 and 322 (sh), 382 ($AlCl_3$ and $AlCl_3$/HCl, λ_{max}/nm) 313 (sh), 366, (NaOAc and NaOAc/H_3BO_3, λ_{max}/nm) 300, 345 and 300, 341. This data are identical with the reported data (Markham and Jaipetch, 1982); FT-IR (KBr, cm^{-1}): 3416 (ν_{OH}), 2972 (ν_{CH}), 1627 ($\nu_{C=O}$), 1573, 1543, 1485 ($\nu_{C=C}$), 1341(($\delta_{ip\text{-}OCH3}$), 1256, 1225, 1210 ($\nu_{as\ C\text{-}O\text{-}C}$), 1112, 1041 ($\nu_{sy\ C\text{-}O\text{-}C}$), 997, 972, 866, 824 ($\delta_{oop\text{-}CH}$) were observed.

alpinetin(compound-6) was colourless needle-shaped crystal from ethanol extract. uv-vis (MeOH and MeOH/NaOH λ_{max}/nm) 285, 315 (sh) and 247, 322 (sh), ($AlCl_3$ and $AlCl_3$/HCl, λ_{max}/nm) 285, 315 (NaOAc and NaOAc/H_3BO_3, λ_{max}/nm) 247, 322 and 288, 316 (sh). This data are identical with the reported data (Markham and Jaipetch, 1982); FT-IR (KBr, cm^{-1}): 3471 (ν_{OH}), 2973, 2939 (ν_{CH}), 1621 ($\nu_{C=O}$), 1581 ($\nu_{C=C}$), 1361, 1318, 1308 (($\delta_{ip\text{-}OCH3}$), 1213, 1182 ($\nu_{as\ C\text{-}O\text{-}C}$), 1110 ($\nu_{sy\ C\text{-}O\text{-}C}$), 837, 769, 702 ($\delta_{oop\text{-}CH}$) were observed.

helichrysetin(compound-7) was colourless needle-shaped crystal from ethanol extract. uv-vis (MeOH and MeOH/NaOH λ_{max}/nm) 269, 360 and 262 (sh) 425, ($AlCl_3$ and $AlCl_3$/HCl, λ_{max}/nm) 258, 342 and 258, 360; FT-IR (KBr, cm^{-1}) : 3394 (ν_{OH}), 3008 ($\nu_{Ar\text{-}CH}$), 2927, 2858 ($\nu_{CH2,CH3}$),1635 ($\nu_{C=O}$), 1601, 1446, 1423 ($\nu_{ar\ C=C}$), 1373 (($\delta_{ip\text{-}CH3}$), 1161 ($\nu_{as\ C\text{-}O\text{-}C}$), 1107, 1061 ($\nu_{sy\ C\text{-}O\text{-}C}$). ^1H NMR (400 MHz, CD_3OD, δppm): 7.59 (1H, H-7, d, J=15.6 Hz), 7.39 (2H, H-3, H-6, d, J=8.5 Hz), 7.34 (2H, H-2, H-6, d, J=8.5 Hz), 6.87 (1H, H-8, d, J=15.6 Hz), 6.1 (1H, H-3', d, J=2.76Hz), 6.05 (1H, H-5', d, J=2.76 Hz), 3.88 (3H, OCH_3, s) were observed.

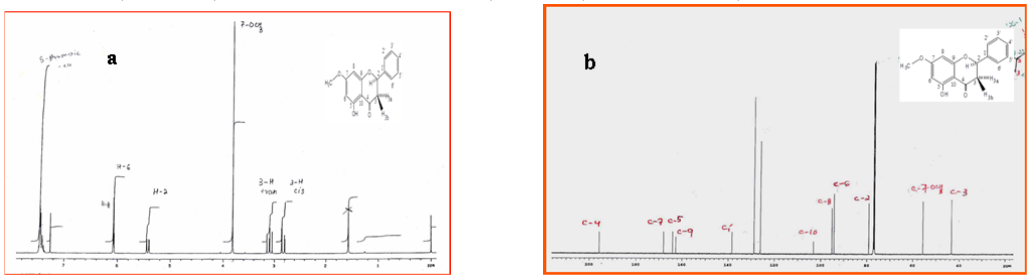

Figure 2. (a) ^1H NMR spectrum of isolated compound A-1 (b) ^{13}C NMR spectrum of isolated compound A-1

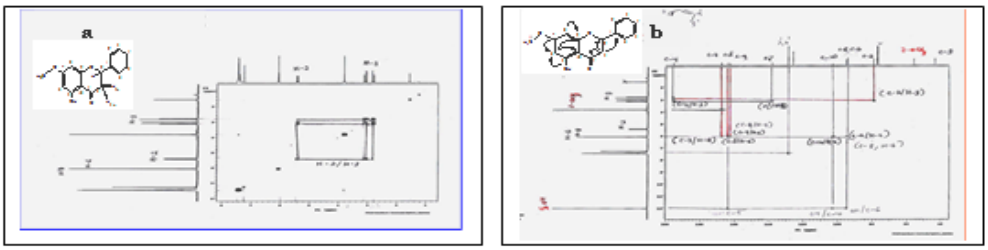

Figure 3.

(a) ^{1}H-^{1}H COSY spectrum of isolated compound A-1 *(b) HMBC spectrum of isolated compound A-1*

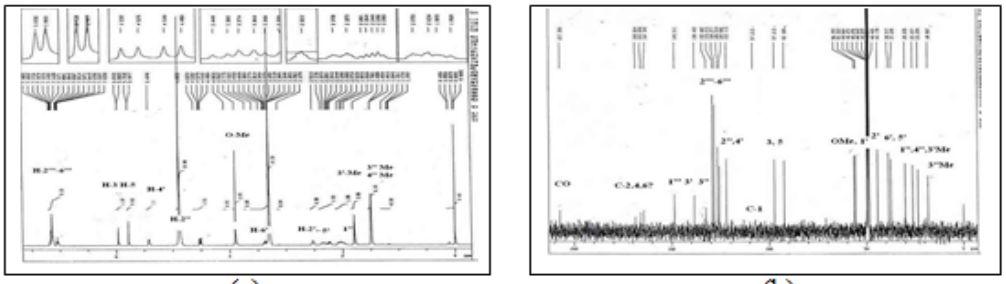

Figure 4.

(a)^{1}H NMR spectrum of compound -2 *(b)^{13}C NMR spectrum of compound-2*

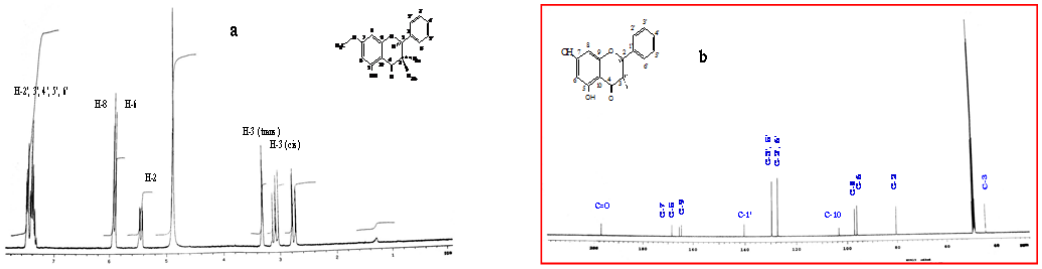

Figure 5.(a)^{1}H NMR spectrum of compound -3 (b)^{13}C NMR spectrum of compound-3

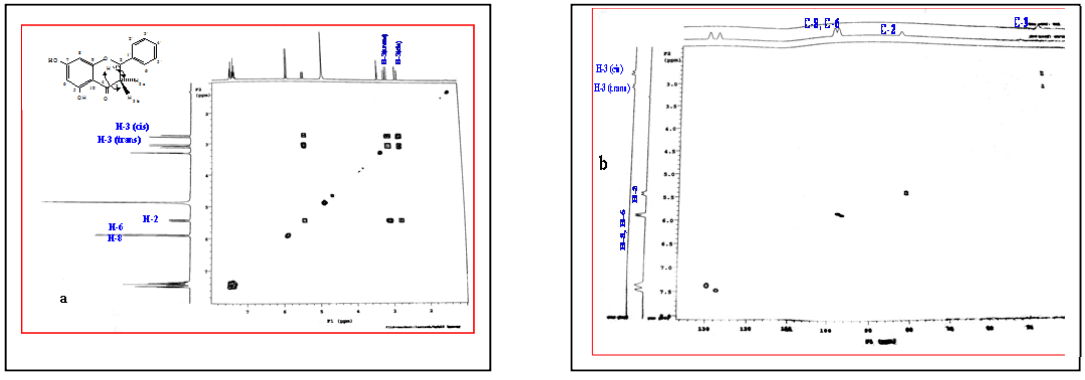

Figure 6. (a^{1}H-^{1}H COSY (300 MHz, CD_3OD) spectrum of isolated compound A-3

(b) HMQC (300 MHz, CD3OD) spectrum of isolated compound A-3

Rapid Screening of Antioxidant by Dot-Blot and DPPH Staining

Ethyl acetate and ethanol extracts from *B. pandurata* (red and yellow) rhizomes were detected in the TLC plates by DPPH staining method. The appearance of yellow spot has potential value for antioxidant activity (Change et al., 2002).In the According to this result, ethyl acetate extract showed the more potent antioxidant activity followed by ethanol extract. Antioxidant activity of isolated compounds from *B. pandurata* (yellow) rhizome was detected on TLC plate by Dot-Blot and DPPH staining. In DPPH staining, compound-2 (panduratin A), compound -3 (pinocembrin), compound-5 (cardamonin) and compound -7 (helichrysetin) showed the radical scavenging activity at lowest amount ranging from 50 μg g to 400 μg g. However, compound compound-1 (pinostrobin) and compound-6 (alpenetin) did not show antioxidant activity (see Figure 3).

4. Conclusions

By silica gel column chromatographic separation technique, seven compounds namely; pinostrobin (5-Hydroxy-7-methoxy Flavanone), panduratin A, pinocembrin (5,7-dihydroxy Flavonone), 2', 6' dihydroxy-4'-methoxy chalcone, cardamonin (4', 6' dihydroxy-2'-methoxy chalcone), alpinetin (7-hydroxy,5-methoxy flavanone) and helichrysetin (4,4',6' trihydroxy-2'methoxy chalcone) were isolated from (yellow) rhizome of *B. pandurata*. The structures of all these compounds were identified and elucidated by Co-TLC method and Joint application of modern spectroscopic techniques. In antimicrobial activity, water, petroleum ether, ethyl acetate and ethanol extracts (red and yellow rhizome) have been tested against on 12 strains of microorganisms by agar disc diffusion method**.** Among four extracts of *B. pandurata* (yellow) rhizome, petroleum ether and ethyl acetate extracts were the more active against 9 stains of microorganism (ID 14-23 mm) than that extract of red rhizome showed potent antibacterial activity against 4 strains of microorganism (ID 14-16 mm). Therefore, *B. pandurata* (Roxb.) (yellow rhizome) may possess higher antimicrobial potency than that of red rhizome. In antioxidant activity, ethanol and ethyl acetate extracts of both rhizomes and isolated compounds were detected in the TLC plates by the DPPH staining method. Ethanol and ethyl acetate extracts of both rhizomes showed potent activity at the dry matter amount (50 μg – 400 μg). Pinocembrin, cardamonin, panduratin A and helichrysetin showed high radical scavenging activity (50 μg-400 μg). 2', 6'-dihydroxy-4' methoxy chalcone showed radical scavenging activity (100 μg – 400 μg). Therefore, the phytoconstituents may contribute significantly to potent antioxidant activity.

Acknowledgements

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Study on Antimicrobial Activity and Antioxidant Activity of Myanmar Traditional Medicine (A-phay-kyaw) as Remedy for food Poisoning

Khin Htwe Kyaing[1], Thida Kyaw[2]

Abstract

In this research work, one of the Myanmar traditional herbal medicine A-Phay-Kyaw (A-P-K) used as remedy for food poisoning was chosen to determine antimicrobial and antioxidant activities. Elemental constituents of sample (A-P-K) were determined by WDXRF spectrophotometer. From the mineral analyze, the results showed the highest percent of calcium and potassium present in APK medicine. In addition, two kinds of drug extract such as ethanolic and actone were tested for their antioxidant activities. Antioxidant activities were performed by DPPH (1-1-diphenyl-2-picrylhydrazyl) radical scavenging method for different extracts of medicine which showed that this medicine on higher concentration possess better antioxidant potential when compare to standard ascorbic acid. Furthermore the antimicrobial activities of the drug extracts were studied by Agar well diffusion method on five tested organisms such as *Eschericha coli, Enterococous faecalis, Staphylococcus aureus, Bacillus cereus and Candida albicans.*

Keywords: WDXRF, DPPH, Antioxidant activities, Agar well

1. Introduction

Traditional medicine comprises medical aspects of traditional knowledge that developed over generations within various societies before the era of modern medicine. In a number of industrialized countries many people regularly use some form of traditional complementary and alternative medicine (TCMA). The World Health Organization (WHO) defines traditional medicine as the sum of the knowledge, skills and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness/ disorders. Traditional medical systems are challenging because their theories and practices strike many conventionally trained physicians and researchers as incomprehensible. The dependence of man on nature for food and medicines goes back to primitive times. The oldest civilizations have indication of plants being worshiped by man and different forms of grain are found associated with human being globally. Initially, man was dependent on plant system for its medical needs vitality and cure of diseases[1]. Around 200 people suffered from food poisoning each year but grateful no-one died. Harmful, ingestible substances can be grouped into three distinct categories, chemical threats, physical hazards and biological contamination. According to nutritionists, most food poisoning in Myanmar is caused by biological contamination such as bacteria or viruses (website-2). A-P-K medicine was prepared by many herbal based materials with significant activity. This medicine was taken easily everybody. This medicine used for the diseases such as diarrhea, diabetic, hypertension, nausea and gastritis due to contaminated food. It is also useful for skin diseases such as allergy and poisonous animals. The drug has been reported that it has

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antidiabetic, antiacid, antidote properties. This medicine has also food digestibility. So, it was selected for investigation of antioxidant activity, antimicrobial activities and elemental contents in this sample.

2. Material and Method

Sample Collection

A-phay-kyaw medicine was purchased from Myanmar Tradition Medicine Shop, Zaycho Market, Mandalay, Myanmar.

Preparation of Sample Powder

Myanmar Traditional drug of A-P-K is made to powder by using blander and the powders were stored in screwed-capped.

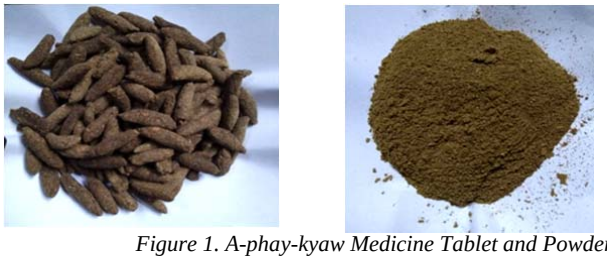

Figure 1. A-phay-kyaw Medicine Tablet and Powder

Determination of pH of the sample

Sample 2g was dissolved into the beaker 100mL of distilled water. It was heated on the hot plate for 15 minutes. After cooling, the solution was filtered. The pH of the filtrate was determined by using PH paper and PH meter.

Extraction of Sample

Preparation of Ethanolic Extract (Cold Method)

100 g of sample was dissolved in 300mL of ethanol. It was stored at room temperature for one month and it was filtered. The solvent was evaporated at room temperature. The ethanolic extract was used to determine the antioxidant and antimicrobial activities.

Preparation of Actone Extract (Cold Method)

100 g of sample was dissolved in 300mL of actone. It was stored at room temperature for one month and it was filtered. The solvent was evaporated at room temperature. The ethanolic extract was used to determine the antioxidant and antimicrobial activities.

Preparation of Watery Extract

Sample 3g was dissolved in 90 mL of distilled water. It was heated in a water bath for six hours and then filtered. The water content in filtrate was evaporated with water bath. The watery extracted of the sample was determined by antioxidant activity.

Determination of Elemental Contents

Qualitative determination for elemental contents of A-P-K was studied at Yadanabon University by applying Wavelength Dispersive X-ray Fluorescence (Rigaku Supermini200-WDXRF) method.

Determination of Antimicrobial Activities

The antimicrobial activities of ethanol, water and actone extracts of medicine were done by Agar well diffusion method on five organisms in Biotechnology Research Department (BRD), Kyaukse, Mandalay Division, Myanmar. The selected organisms are *Eschericha coli, Enterococous faecalis, Staphylococcus, Bacillus cereus and Candida albicans.*

Determination of Antioxidant Activities

In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals is monitored. The sample extract (0.2g) is diluted with ethanol and (2.5 mL) DPPH solution is added. After 30 mins, the absorbance is measured at 517 nm by using method[3]. (Manzocco *el al.*, 1998). In this experiment three solutions were prepared, DPPH solution, standard solution, and various concentration of sample solution.

Calculation of Half Maximal Inhibition Concentration

IC_{50} values were obtained from the best- fit line plotted concentration versus absorbance. Ascorbic acid was used as a positive control in the bioassay. IC_{50} value was calculated by using linear regressive equation[2].

3. Results and Discussion

Elemental Contents of Myanmar Traditional herbal based medicine (A-P-K)

Table1. Mineral Content of A-P-K by WDXRF

No.	Element	Symbols	Concentration (%)
1.	Calcium	Ca	38.6
2.	Potassium	K	23.4
3.	Chlorine	Cl	18.2
4.	Silicon	Si	5.41
5.	Magnesium	Mg	4.54
6.	Iron	Fe	3.86
7.	Phosphorus	P	2.16
8.	Sulfur	S	1.90
9.	Aluminum	Al	1.38
10.	Manganese	Mn	0.416

Table (1) shows the high percent of some minerals were found in sample. Among them, calcium is the highest amount in the sample and the second is potassium content. The cation's contribution to blood pressure regulation is most prominent in its influence on vascular resistance.

pH, Moisture and Ash analysis

Table 2. pH values of medicine(A-P-K)

No. of experiment	pH
1	7.34
2	7.32
3	7.34

A-P-K medicine has slightly basic character and traditional physician describe this type of medicine is used as cold/hot medicine. The moisture content of medicine changes with the weather. Myanmar traditional medicine (A-P-K) can absorb the moisture from its environment. The moisture content of A-P-K was found to be 6.52 to. 6.72 %.

Table 3. Moisture content of medicine(A-P-K)

No. of experiment	Wt of sample (g)	Wt of loss (g)	Moisture (%)
1	1.00	0.652	6.52
2	1.00	0.661	6.61
3	1.00	0.672	6.72

The ash content is a measured of the total amount of minerals present within a drug, whereas the mineral content is a measured of the amount of specific inorganic components present in medicine such as Ca, Na, K, Cl.(websute-3)

Table4. Ash content of medicine (A-P-K)

No. of experiment	Ash content (%)
1	29.01
2	29.02
3	29.00

Antioxidant Activity

Ascorbic acid was used as a positive control for DPPH radical scavenging assay. IC_{50} value of Ascorbic acid is 84.78 μg / mL.

Table 5. % inhibition of various concentration of Sample (A-P-K)

Sample concentration	**Mean % inhibition**	**IC_{50} (µg/mL)**
100	70.03	60.37
90	61.60	
80	58.58	
70	56.71	
60	53.79	
50	46.30	
40	28.71	
30	15.12	

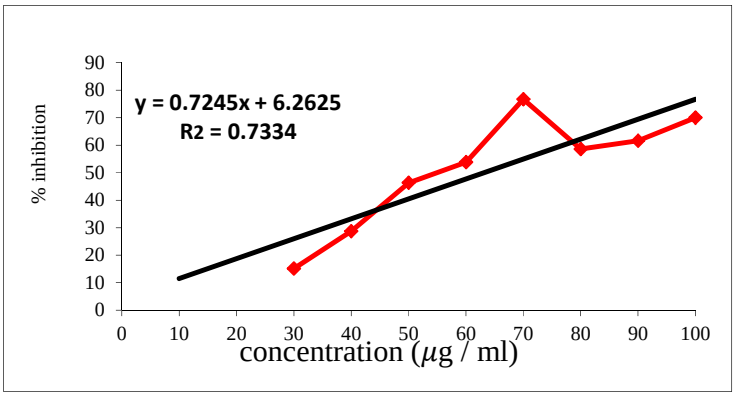

Figure3. % Inhibition in Different Concentration (EtOH Extract)

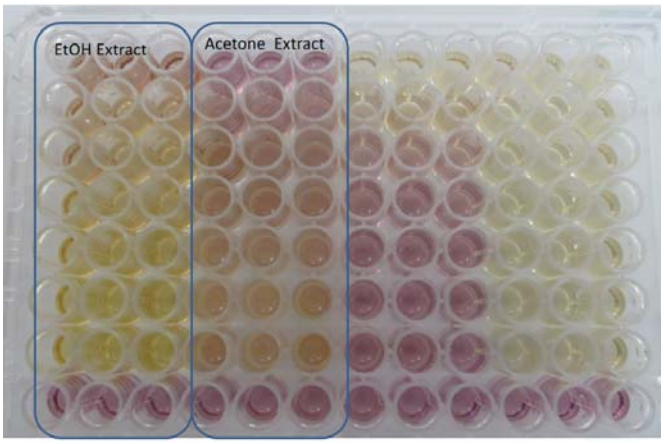

Figure4. Test for Antioxidant Activity of ethanol and actone extracts of A-P-K

Antimicrobial Activity

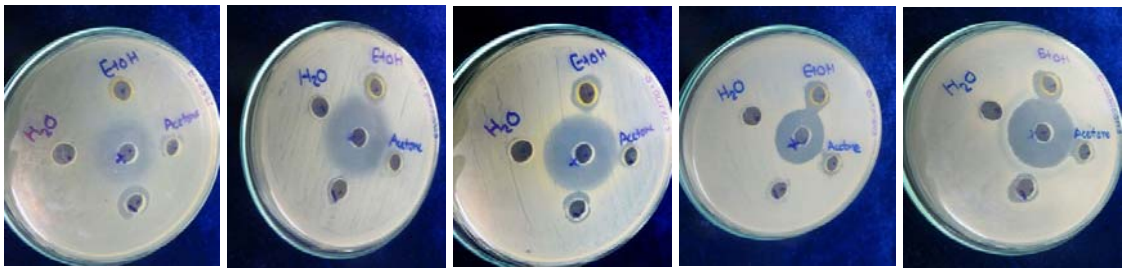

Figure5. Antimicrobial Activities of Medicine (A-P-K)

Table 6. Antimicrobial Activities of Medicine (A-P-K)

Sample	Inhibition zone diameter (mm)				
	Escherichia coli	***Enterococcus faecalis***	***Staphylococcus aureus***	***Bacillus cereus***	***Candida albicans***
Water Extract	0	0	0	0	0
Ethanol Extract	11	13	14	13	15
Acetone Extract	12	12	10	12	12

Agar well – 8mm

The inhibition zones of various extracts are shown in table.4. It was clearly noted that ethanol and actone extract of sample possessed antimicrobial activity against all strains tested. Especially, ethanol extract was more active against *Candida albicans* and *Staphylococcus aureus* species.

4. Conclusion

The result from PH analysis indicates A-P-K have slightly basic character. The moisture content of the medicine can change with the weather. Myanmar traditional herbal based medicine can absorb the moisture from its surroundings. So the medicine should be carefully stored to maintain the quality of medicine. From the qualitative determination of elemental contents of this medicine was known that 38.6% of calcium, 23.4% of potassium, 18.2% of chlorine, 5.41% of silicon, 4.54% of magnesium and 3.86% of iron include in the sample. Among these elements, the amount of calcium and potassium were the highest percent in the sample. Calcium is important to bone growth and formation, blood clotting, nerve and muscle functioning. Potassium is one of the seven essential macro-minerals. The human body requires at least 100 milligrams of potassium daily to support key processes. It also decreases the risk of stroke and lower blood pressure (websit-1). The antimicrobial activities of the medicine of A-P-K were tested by Agar-well diffusion method on five tested organisms. It is clearly noted that ethanol and actone extract of sample possessed antimicrobial activity against all strains tested. Especially, ethanol extract was more active against *Staphylococcus aureus* and *Candida albicans* species than other. But, water extract was not active against on all organisms. The lower the IC_{50} value shows the higher radical scavenging activity. According to the antioxidant activity, IC_{50} value of

A-P-K was found to be 60.37 μg/mL. Ascorbic acid was used as a standard and the IC_{50} value was 84.78 μg/mL. Therefore, the ethanol extract of sample is higher antioxidant activity than standard ascorbic acid.

So, A-P-K medicine should be used safety for abdominal pain due to infected with disease by eating improperly prepared or unclean food/ contaminated food. Further investigation the cyto-toxicity of this medicine by using in vivo method should be pursued.

Acknowledgements

I would like to express our profound gratitude to Dr Maung Maung Naing (Rector, Yadanabon University), Dr Si Si Khin (Prorector, Yadanabon University) , Dr Tint Moe Thu Zar (Prorector, Yadanabon University), and Dr Hlaing Hlaing Myat, (Professor and Head of Chemistry Department, Yadanabon University) for their suggestion and permission. We also convey special gratitude to chairperson and committee members of SCA 2019. Myanmar.

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Online Materials

1. http://www.medicalnewstoday.com>art
2. http//definedterm.com
3. https://www.mmtimes.com

The Role of Career Development for Employee Job Satisfaction

Khin Marlar Maung[1]

Abstract

The strong competitive environment demands both manufacturing and service organizations to attract skillful and talented employees for long-term success. The study explored the effects of career planning and career motivation on career development and employee job satisfaction. Several strategies including career development are applied to attract and retain the key employees. As the antecedents of career development, career planning and career motivation are considered to develop different competencies of employees. As main analysis, Structural Equation Modeling (SEM) with Linear Structural Relationships (LISREL) 8.72 is utilized. The sample of 206 employees from Nature Timber Trading (NTT) Co., Ltd. manufactured plywood in Mandalay was selected as the respondents. The results reveal that career planning and career motivation have direct and significant effects on career development and career development has direct and significant effects on employee job satisfaction. The importance of career development and its antecedents should be emphasized by organizations to upgrade employee job satisfaction. Empirical research on career development has not been conducted among the plywood factories in Mandalay.

Keywords**:** career planning, career motivation, career development, job satisfaction

1. Introduction

In the strong competitive business environment, attracting and retaining talented and skillful employees is essential for success of any organization. Human resources managers must set actions and decisions to upgrade the long-run performance of business. Among the practices, career development is strongly demanded in improving employee satisfaction and performance. Employees with strong career insight would be likely to engage in trying to obtain career opportunities and establishing realistic goals and action plans to upgrade their level of career satisfaction [1].

This study aims to fulfill the human resource management literature by investigating the importance role of career planning and career motivation and their association with career development and employee job satisfaction of Nature Timber Trading (NTT) Co., Ltd. in Mandalay, Myanmar. The specific objectives are to analyze (a) the effects of career planning and career motivation on career development and job satisfaction, (b) the effects of career development on job satisfaction and (c) the mediating effect of career development.

Career planning means matching individual strengths and weaknesses with occupational opportunities and threats [2, p.328]. [3] also defined career planning as an ongoing process whereby an individual sets career goals and identifies the means to achieve them. The important of career planning for career development and job satisfaction is demanded to be explored. The following hypotheses are tested to prove the role of career planning.

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H1a: Career planning has direct effect on career development.

H1b: Career planning has direct effect on job satisfaction.

The career motivation theory is used to understand career related attitudes and behavior of employees. Career development theories attempt to explain various careers and work related behaviors to fit the present job and career goals. The following hypotheses are tested in this study to prove the effects of career motivation on career development and job satisfaction.

H2a: Career motivation has direct effect on career development.

H2b: Career motivation has direct effect on job satisfaction.

According to [3], career development is to ensure that people with the proper qualifications and experiences are available when needed. Career development is the lifelong series of activities that contribute to a person's career exploration, establishment, success, and fulfillment [2]. Based on the results of previous studies, the present study tests the effects of career development on employee job satisfaction and the mediating role of career development by the following hypotheses.

H3: Career development has direct effect on job satisfaction.

H4: Career development mediates the relationship between career planning, career motivation and job satisfaction.

Job satisfaction is defined as "the extent to which people like (satisfaction) or dislike (dissatisfaction) their jobs" [4]. Additionally, job satisfaction will vary directly with the needs of individual employees and their expectation to satisfy their needs. According to [5], career development is one of the essential requirements related to the job of employee and it creates job satisfaction. In the present study, the different effects of career planning, career motivation and career development on job satisfaction are explored to fulfill the research gap of plywood factory in Mandalay, Myanmar.

2. Methods

Structured questionnaire was used to collect primary data. In this study, 27 measured variables are included. According to [6], the required sample size is at least 270 and questionnaires were distributed to the selected sample from Nature Timber Trading (NTT) Co., Ltd. manufactured plywood in Mandalay. 206 complete set of questionnaires were included in the final analysis of the study. The response rate is 76.29%. Structural Equation Modeling (SEM) with Linear Structural Relationships (LISREL) 8.72 was used as the analytical tool to test the results of the proposed hypotheses. Confirmatory factor analysis (CFA) was carried out in order to validate the relationship among the measurement variables and the latent factors in the model. Some items were deleted to meet the reliability analysis.

3. Results and Discussion

The results of the variables exceeded the minimum acceptable reliability level are shown in Table 1.

Table1. Means, reliability and correlations of variables

Variables	Mean	CP	CM	CD	JS	Alpha	Item
Career planning (CP)	3.72	_				.843	6
Career motivation (CM)	3.79	.331**	_			.871	6
Career development (CD)	4.11	.705**	.726**	_		.896	7
Job satisfaction (JS)	3.98	.585**	.491**	.773**	_	.901	6

***. Correlation is significant at the 0.01 level (2-tailed);*

Source: Survey data (May, 2019)

As the first stage of analysis, model fit statistics was tested. The model fit was assessed through multiple fit indexes: based on the values of χ^2/df; comparative fit index (CFI); incremental fit index (IFI); normed fit index (NFI); Non-normed fit index (NNFI); root mean square error of approximation (RMSEA) and standardized root mean square residual (SRMR) [7] [8]. The proposed model was measured with twenty seven items for all latent variables. Because of the poor model fit statistics, the revised model with twenty five items showed the acceptable model fit statistics in Table 2.

Table 2. Models and fit statistics

Models	χ^2	df	CFI	IFI	NFI	NNFI	SRMR	RMSEA
Proposed model	283.96	74	0.92	0.93	0.93	0.92	0.11	0.15
Revised model	63.48	42	1.00	1.00	0.99	0.99	0.03	0.02
Suggested values	χ^2/df < 2		≥ 0.95	≥ 0.95	≥ 0.95	≥ 0.95	≤ 0.08	≤ 0.05

All χ^2 values are significant at $p < 0.05$. df = degree of freedom,

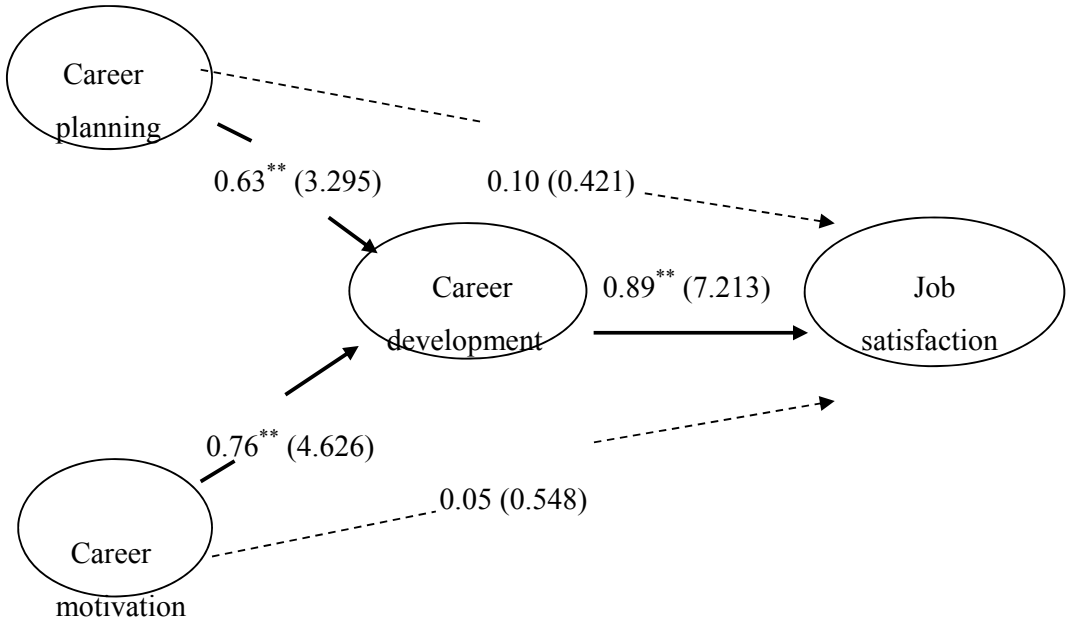
The results revealed that career planning had significant direct effect on career development (γ = 0.63, t = 3.295). Career motivation had significant direct effect on career development (γ = 0.76, t = 4.626). Career development had significant direct effect on job satisfaction (β = 0.89, t = 7.213). The final results of the structural model with direct, indirect and total effects of the latent variables are shown in Table 3 and Figure 1.

Table 3. Summary of effects

Variables	Career development			Job satisfaction		
	DE	IE	TE	DE	IE	TE
Career planning	0.63**	0.04	0.67**	0.10	0.53**	0.63**
Career motivation	0.76**	0.01	0.77**	0.05	0.69**	0.74**
Career development	-	-	-	0.89**	-	0.89**

Source: Survey data (May, 2019)

**p < .01; DE = Direct effect; IE = Indirect effect; TE = Total effect



P< 0.05

Figure 1: Results of structural model (t value with bracket)

According to the results, hypothesis 1(a), 2(a) and 3 were supported. On the other hand, there is no significant direct effect of (a) career planning on job satisfaction and (b) career motivation on job satisfaction. Hypothesis 1(b) and 2(b) were not supported. According to the results of hypothesis 1(a), 1(b), 2(a), 2(b) and 3, it is concluded that career development full mediated the relationship between career planning, career motivation and job satisfaction. According to the mediating result, hypothesis 4 was supported.

The objectives of this study are to explore the effects of career planning and career motivation on career development and job satisfaction and the mediating role of career development between career planning, career motivation and job satisfaction. The correlation results revealed that career planning and career motivation have strong correlation with career development and career development has strong correlation with job satisfaction. It is concluded that the more the company effectively practice career planning and motivation, the more the career development and job satisfaction can be yielded as the outcomes. The effective career plan

for employees and motivation should not be ignored for career development and job satisfaction of employees. The direct effect of career motivation on career development is more than career planning. The results showed that the employees expect valuable and attractive career motivation to learn more about the job-related knowledge. The company should review career plan to be effective for career development.

On the other hand, the direct effects of career planning and career motivation on job satisfaction cannot be explored in this study. The importance of these two latent variables in the workplace is the area to be reviewed to gain job satisfaction. The effective implementation of career planning and career motivation is not only for career development but also for job satisfaction of employees. The organization should review the awareness of employees about career plan and motivation.

The direct and significant positive effect of career development on job satisfaction was also explored in this study. The result supports findings of previous empirical studies. The successful career development activities attract employees and create job satisfaction. The absent of career development at job will create negative perception of employees on job satisfaction. Thus, the role of career development should not be ignored by organizations because job satisfaction depends on career development.

The mediating effect of career development is also explored in this study. Career development fully mediated between career planning, career motivation and job satisfaction. It is proved that career planning and career motivation are not enough to create job satisfaction without career development. This study shares the knowledge that career planning and career motivation are the antecedents of career development and also job satisfaction is the outcome of career development.

4. Conclusion

In conclusion, job satisfaction of employees is expected through career development. Career development is influenced by career planning and career motivation. The results revealed that career planning and career motivation have significant effects on career development and career development creates job satisfaction. Moreover, career development is a full mediator and thus, career planning and career motivation are the requirement for job satisfaction through career development. It can be concluded that human resources managers need to find the best and most possible ways to have career plan with the awareness of employees and to support career motivation among their employees among employees to yield job satisfaction and performance improvement.

The limitations of the study will be fulfilled by further research. In this study, as one limitation, career planning and career motivation are only selected as the two factors to shape career development. The other significant factors such as job rotation, coaching, mentoring, training, etc. can create career development. Another limitation is that primary data was collected only from Nature Timber Trading (NTT) Co., Ltd. located in Mandalay. Different manufacturing and service organizations from other areas should be tested. Further research will provide valuable and unexpected results to improve career development and job satisfaction of employees.

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Natural Water Filter by Using Plant Xylem from Pine Tree and H_2S Paper-Strip Test

Khin Lay Thwe[1], Su Su Hlaing[2], Hone Bu[3]

Abstract

A simple natural water filter is constructed by using plant xylem from pine tree. The plant xylem tissue is the most suitable for filtration. Plant xylem from the pine trees – a readily available, inexpensive, biodegradable and disposable material – can remove bacteria from water by simple method. And the filtered water quality is examined by the low-cost hydrogen sulphide (H_2S) paper-strip test which is simple to use and easy to interpret. It indicates whether the presence or absence of faecal coliform bacteria in the sample after filtration and the filtered water is safe to drink or not. In the present work, the microbiological and chemical water quality of water samples from the lake, tap water and purified drinking water are tested.

Keywords: Natural water filter, Plant xylem, (H_2S) paper-strip test, Faecal coliform bacteria

1. Introduction

Effective point-of-use devices for providing safe drinking water are urgently needed to reduce the global burden of waterborne disease. Plant xylem from the sapwood of pine trees – a readily available, inexpensive, biodegradable and disposable material can remove bacteria from water by simple filtration. Approximately 3 cm^3 of sapwood can filter water at the rate of several liters per day, sufficient to meet the clean drinking water needs of one person. The results demonstrate the potential of plant xylem to address the need for pathogen-free drinking water in developing countries and resource-limited settings. A small piece of sapwood can filter out more than 99 percent of the bacteria E. coli from water. The size of the pores in sapwood - which contains xylem tissue evolved to transport sap up the length of a tree - also allows water through while blocking most types of bacteria [1].

But micro-biological and chemical testing of drinking water quality should be performed to indicate whether the water is safe to drink or not. Typically, the key water quality parameter that indicates safety is the absence of faecal coliform bacteria in the sample [2]. An alternative low-cost test for faecal contamination in drinking water which is simple to use and easy to interpret is the hydrogen sulphide (H_2S) paper-strip test. There are many advantages of this test for use in rural and remote communities particularly where conventional monitoring is not possible or too expensive. So in the present work, a simple, low-cost and useful natural water filter is constructed by using plant xylem from pine tree. The three water samples: lake water, tap water and purified drinking water are examined by using low-cost hydrogen sulphide (H_2S) paper-strip test before and after filtration to indicate whether the water is safe to drink or not.

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2. Methods

2.1 Construction of a Natural Water Filter

The xylem filter device was constructed by simply peeling off the bark from the pine branch shown in Figure 1(a). Then 1 inch-long section was cut from a pine branch with 1.5 cm diameter. The section was soaked in 10 ml of deionized water before the experiment. Care should be taken to avoid drying of the filter. Wood section was inserted into the end of 1.6 cm internal diameter PVC tubing, sealed with epoxy, secured with hose clamp and allowed to cure for ten minutes before the experiment as shown in Figure 1(b), (c) and (d). In the present study, the three water samples: lake water, tap water and purified drinking water were used to test. The water sample was poured into the tube and flow down through the xylem filter. The filtered water was collected in a bottle and sealed it to test. The water samples were examined by using H_2S paper-strip test before and after filtration with plant xylem filter. The water filtration set up by using plant xylem natural water filter was shown in Figure 2(a), (b) and (c).

(a) *(b)* *(c)* *(d)*

Figure 1. (a) Pine branch (b) 1 inch-long cut section (c) Soaking in deionized water and (d) Fastening in the tube

(a) *(b)* *(c)*

Figure2. Water filtration set up by using plant xylem filter

The H_2S Paper-Strip Test

The test tubes were first cleaned by washing in detergent, rinsing with tap water and drying in air. The medium used in the test was prepared from the following chemicals, as shown in Table 1, which were dissolved into distilled water. Then the mixture was stirred to dissolve the chemicals. To make the paper-strip, 99.9% germ-free Cellox tissue was used to place a measured quantity of media onto the paper. Each paper strip for a 10 ml test sample needs to contain 0.5 ml of media (50 ml sample will use 2.5 ml of media and a 100 ml sample will require 5 ml of media etc.). The Cellox tissue was cut to a size that has absorbed 0.5 ml of media. Then the paper strip was wet by 0.5 ml of media. The strip was dried in the sunlight for 30 min. These reagent-impregnated strips can be stored dry (in an envelope or preferably a zip-locked bag) for several months – until ready for use. Before conducting the test, a strip or strips were introduced into the appropriate clean sample bottle. The tubes or bottles should be stored in a dark place until ready for use. It was found that they can be stored for at least 5 years in this manner. The steps for preparation of H_2S paper-strip is shown in Figure 3.

After sampling, the test sample was placed in a dark place for a few days. Every 12 hours examine the samples for changes in color. The observations were recorded as follows: (–) = no change; (+) = slight change, the paper strip or water has turned gray; (++) = the paper strip is partially black; (+++) = the strip and the water sample itself are noticeably black [3]. As noted above, a color change indicates the presence of bacteria of faecal origin. The speed of the reaction will determine the density of organisms present; i.e. the quicker the reaction the higher the number of faecal organisms presence. This can also be interpreted in terms of a risk factor. For example, a slight color change (+) on day three indicates a lesser risk than a strong (+++) change on day 1.

Table1. The chemicals used in preparing the medium

1	Bacteriological peptone	20 g
2	Dipotassium hydrogen phosphate	1.5 g
3	Ferric ammonium citrate	0.75 g
4	Sodium thiosulphate	1 g
5	Liquid detergent	1 ml
6	Distilled water	50 ml
7	Citrate	1 g

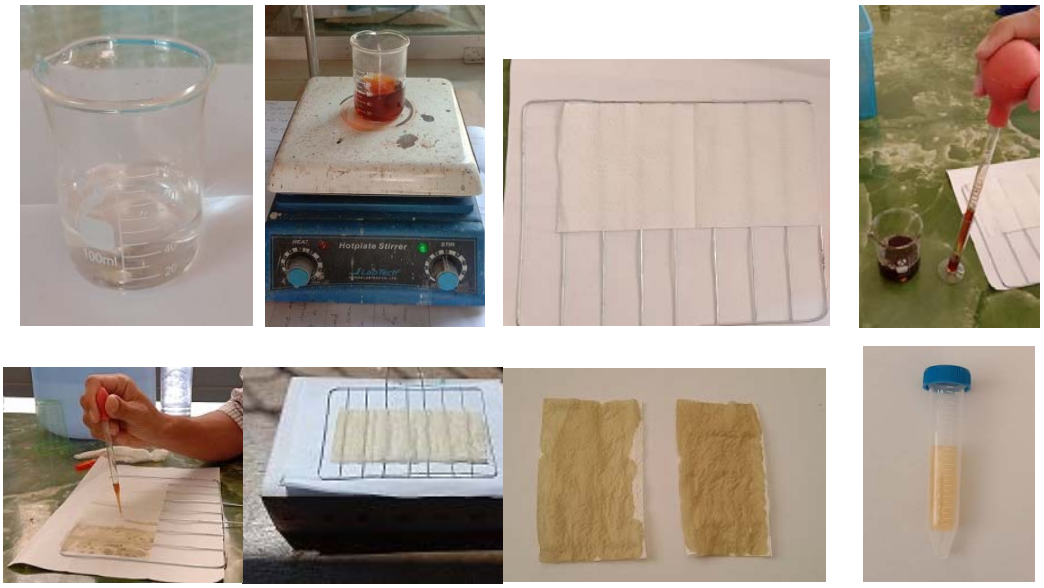
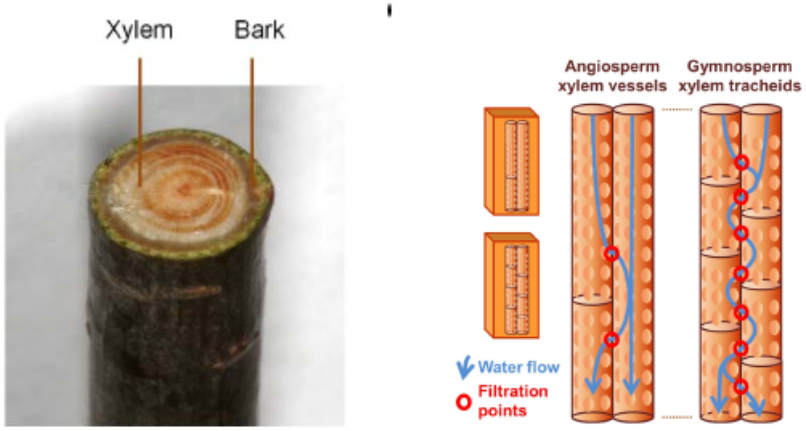


Figure 3. The preparation of H_2S paper-strip

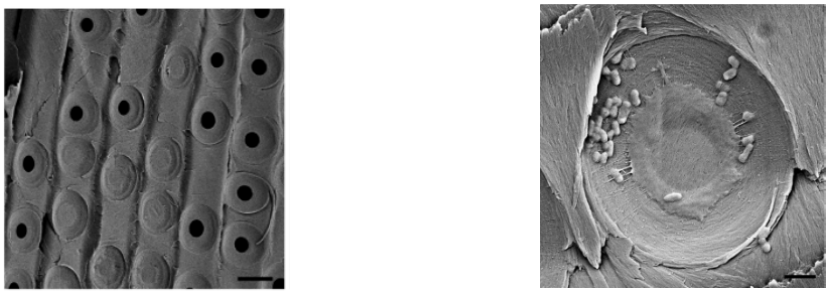
3. Results and Discussion

In woody plants, the xylem tissue is called the sapwood, which often surrounds the heartwood and is in turn surrounded by the bark as shown in Figure 4(a). The xylem structure is shown in Figure 4(b). Figure 5(a) is the SEM image showing pits and pit membranes and Figure 5(b) is the SEM image showing bacteria accumulated on the pit membranes after filtration. But these xylem filters could not filter out 20 nm nanoparticles, which is a size comparable to that of the smallest viruses. It will be interesting to explore whether there exist any coniferous species that have pit membranes with smaller pore sizes that can filter out viruses.



(a) *(b)*

Figure 4. The Xylem structure



(a) ***(b)***

Source: Department of Mechanical Engineering, Massachusetts Institute of Technology, USA. [1]

Figure 5. (a) SEM image showing pits and pit membranes. Scale bar is 20 μm and ***(b)*** *SEM images showing bacteria accumulated on the pit membranes after filtration*

An alternative low-cost test for faecal contamination in drinking water which is visual and simple for people to understand is the hydrogen sulphide (H_2S) paper-strip test. If the sample contains hydrogen sulphide producing organisms, the pad and water turn black. With such an indicator it is not difficult to convince uneducated villagers that the water may not be safe to drink [4]. The hydrogen sulphide (H_2S) paper-strip test is the presence/absence test. The H_2S test just indicates whether there is a risk, not the degree of risk. However, the speed of the reaction (color change from clear to black) indicates bacterial density. The faster the reaction, the greater the numbers of organisms present.

Figure 6 shows the H_2S testing of three water samples before and after filtration with plant xylem filter. Before filtration, the color of lake water and tap water samples turned black on day 2 and day 3 respectively. It means these water were not safe to drink. At that time the purified drinking water sample turned grey only on day 5. It showed that the density of bacteria is more in lake water and the tap water than purified drinking water. So care should be taken not to drink directly the lake or tap water without purification. After filtration with plant xylem filter, it was found that all the water samples were pathogen free and it is safe to drink. Therefore, plant xylem can be used as a natural water filter.

Before filtration

After filtration

(a) Lake Water (b) Tap Water (c)Purified Drinking Water

Figure6. H_2S testing of water samples before and after filtration with xylem filter

4. Conclusions

Plant xylem is a porous material with membranes comprising nanoscale pores. Xylem from the sapwood of pine trees is suitable for disinfection by filtration of water. The xylem filter could effectively filter out bacteria from water. It was learnt that the natural plant xylem water filter can trap any bacteria and producing fresh, uncontaminated water. So, sapwood is a promising, low-cost and efficient material for water filtration, particularly for rural communities where more advanced filtration systems are not readily accessible. The hydrogen sulphide (H_2S) paper-strip test is an alternative low-cost test for faecal contamination in drinking water which is visual and simple for people to understand. If the sample contains hydrogen sulphide producing organisms, the pad and water turn black. The black color and the rotten egg smell of hydrogen sulphide clearly indicate that there is a problem. The three water samples: lake water, tap water and purified drinking water are examined by low-cost hydrogen sulphide (H_2S) paper-strip test before and after filtration with plant xylem. It was found that the density of bacteria is more in lake water and the tap water than purified drinking water. So care should be taken not to drink directly the lake or tap water without purification. After filtration with plant xylem filter, it was found that all the water samples were pathogen free and it is safe to drink. Therefore, plant xylem can be used as a natural water filter.

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Morphodynamics Modelling in a Data-Scarce Environment: The Chindwin River

Khin Kyu Kyu[1], Khin Kyi Cin Linn[2]

Abstract

The Chindwin River is the major tributary of Ayeyarwady River. It is rich in natural resources and is also often the only means of transport connecting the upper and central regions of Myanmar. Understanding the morphdynamics processes of this river would be useful to devise better strategies for the improvement of navigational channel and the prevention of severe river-related problem. Studies on the nature of this river are also facing with problems of data insufficient. The objective of this present paper are to identify the main causes of the problem in the Chindwin River and propose potential solutions. A sediment transport model is designed using not only the currently available dataset but also making numerical assumptions. The analysis is confirmed by the applications of several modelling packages including Delft3D, QGIS, and HEC-HMS. From the simulation and results, morphodynamics patterns of the Chindwin River can be explained, main causes of sediment budgets are mentioned and the proper solution may be explored. This paper will be able to support in future research works such as navigation channel improvement, land use/cover changes and flood protection which play as important roles in the integrated water resource management of the Chindwin River Basin.

Keywords: The Chindwin River, morphodynamics, sediment transport, modelling packages

1. Introduction

The Chindwin River, the most dominant tributary of the Ayeyarwady River, belongs to a rich natural resources. It plays not only as a vital passage for transportation connecting the upper and central region of Myanmar, also as a source of water for drinking, domestic, agriculture, aquaculture and livelihoods. Morphodynamics of this river have changed seasonally and influenced by tropical monsoon climatic condition, in addition to the result of rapid economic development. Only a very few research works have been done in this river compared with others because of the scarcity of data and being difficult to attain.

2. Methods

The land use/ cover and soil type were analyzed by using Google Earth Engine (GEE), and the part of navigation channel was examined by Delft-3D, QGIS and HEC-HMS. A 30x30 morphologic grid was created in Delft-3D based on the land boundary of the Chindwin River. Bathymetry of the selected channel was digitized to be able to use in the software. All the depth points used in the software were triangular interpolated and other parameters such as viscosity and roughness were assumed as default. Time frame for simulation was set as for only one week and the initial discharge was 1750 m^3/s.

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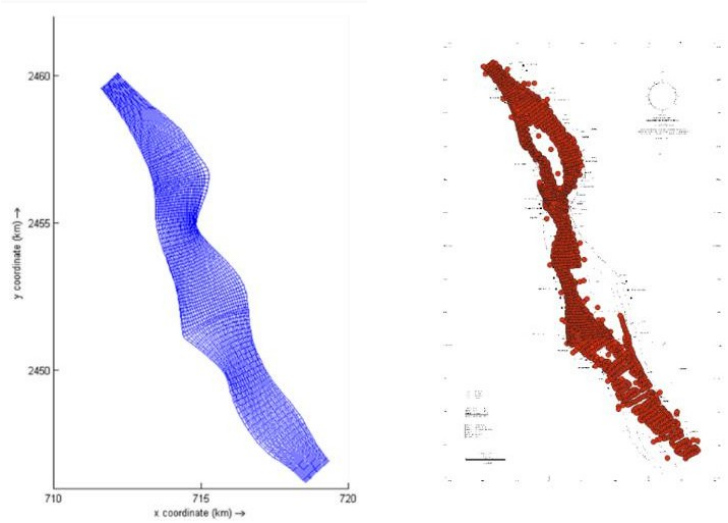


Figure 1. Morphologic grid and digitized bathymetry of Alone Waterway in the Chindwin River

3. Results and Discussion

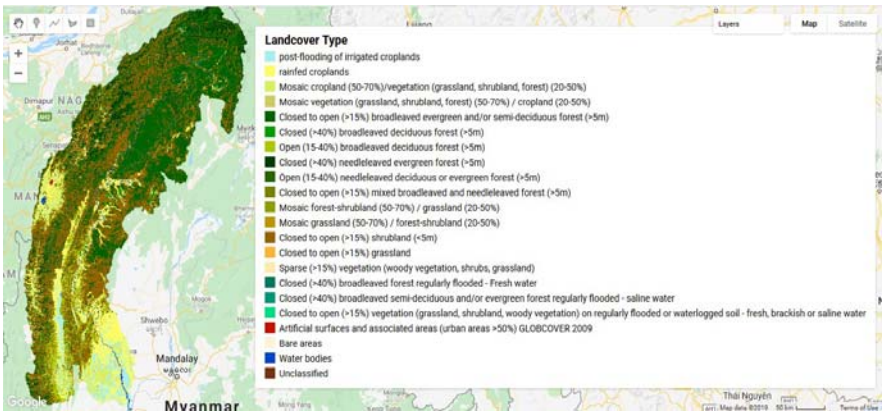


Figure 2. Land cover types of the Chindwin River Catchment (July, 2019) using GEE

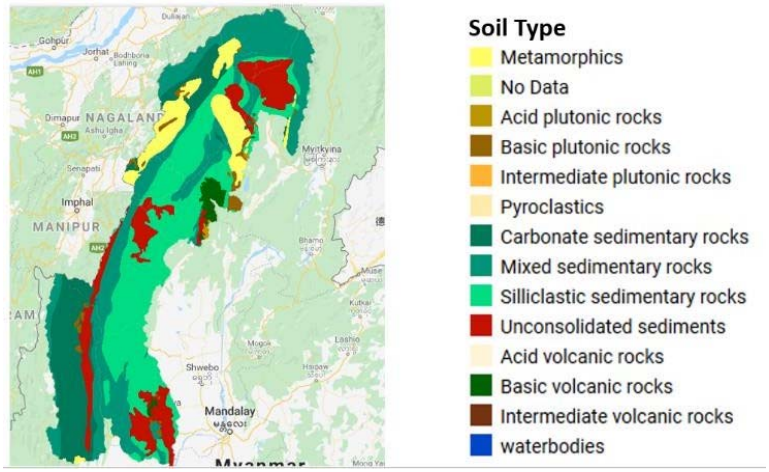
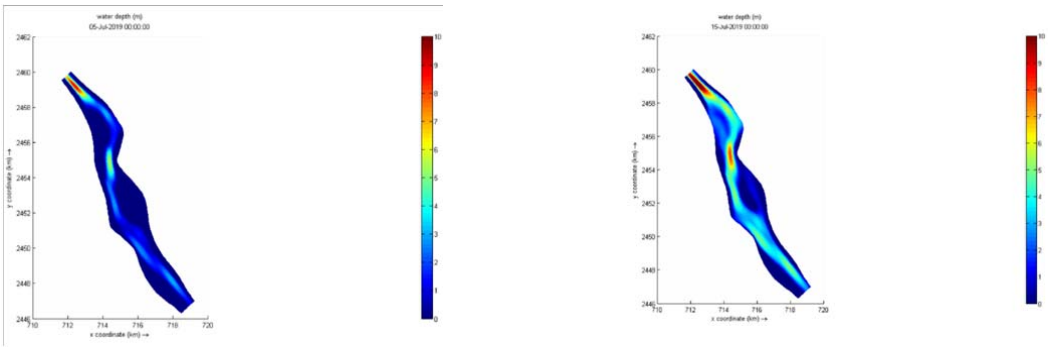


Figure3. Land cover types of the Chindwin River Catchment (July, 2019) using GEE

According to figure 2 and 3, the research showed that the basic geographic and topology along the Chindwin River makes itself to be sensitive with the high flow. The strength of sedimentary rock is originally weak that may lead to the bank and bed of the river more erodible and instable. That can be determined as the main point of the source of landslide during heavy rain in the Chindwin River Catchment.

Moreover, other researches showed that the Chindwin River have been faced with the economic development which causes the severe deforestation in the whole catchment area. This is also the focal problem source of the morphologic problems, such as sedimentation and erosion and navigation problems along the river.



(a) *(b)*

Figure4. (a)Initial water depth, (b) Water depth after simulation for one week

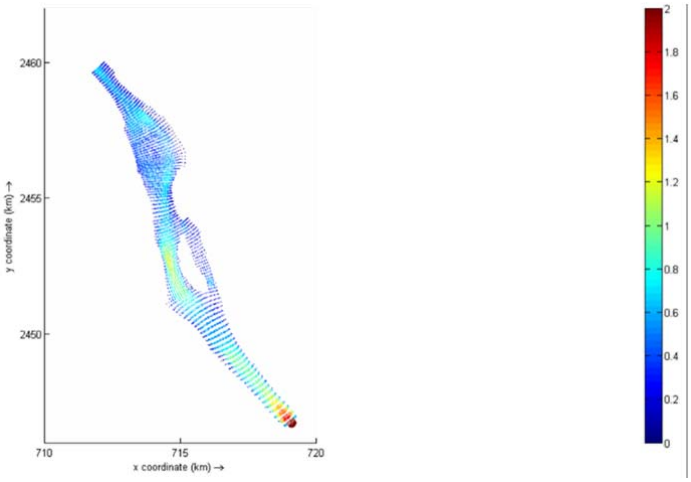


Figure5. Depth-averaged velocity after simulation for one week

The results showed that for only the discharge of 1750 m^3/s, the velocity near eroded bank was running between 0.6-1 m/s. The highest velocity was 1.2-1.4 m/s and it was located at the confluence with North Yama Stream but did not effect on the sand bar. Dredging process was proposed by DWIR and the model should be run again with the resultant water depth.

4. Conclusion

This is the research paper using Delft-3D FLOW resulting only the depth, flow magnitude and direction. Simulation should be included other parameters such as sediment.

Acknowledgements

The author would like to express wholeheartedly her voluminous gratitude everyone who kindly and warmly heaped in writing this research paper. The author would like to pay her unassuming homage to her respectful teachers Dr. Khin Kyu Kyu, Dr. Htike Htike and Dr. Kyi Kyi Lwin who always keeps an eye on her progress and shares invaluable instructions about the research.

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Spectral Analysis of Transition Metal Carbides for Sustainable Industrial Development

Thi Thi Lay[1] et al

Abstract

Surface properties of various transition metal carbide (T-M-C-) materials were analyzed using X-ray photoelectron spectroscopy (XPS). XPS spectra were accumulated according to ISO standards measurement procedures and techniques such and binding energy calibration and sample cleaning. Among (T-M-C) materials, VC, HfC and Cr3C2 spectra data were compare taking into an account the effect of Ar+ ion sputtering in cleaning process. Single crystal VC (100) as well as sintered samples VC, HfC and Cr3C2 were used in the experiment for comparing the differences of sputter effect. Relative intensity ratio in atomic concentration of C/M was compared for sputter effect. The relative intensity ratio(C/M) showed constant nature and the effect of remained surface contaminated species such as oxide were not observed after saturated sputter time. The Ar+ ion sputtering method can be use as practical tools as simple method for sample cleaning in analysis of XPS. The instrument binding energy calibration was carried out according to ISO standards and calculated ΔEcorrection value was -0.6796eV. This correction values was used in calibration of the measured data and photoelectron peaks were well matched with reference data.

Keywords: Transition metals carbides (T-M-C), XPS, Spectral Analysis,

1. Introduction

Development of transition metals carbides (T-M-C) in industrial become importance due to its unique properties such a s hardness, metallic conductivity and high temperatures capability [1, 2, 3, 4]. There have been widespread in applications of T-M-C compounds as catalysis, hard coatings, wear-resistant components and electron emitter devices in car industry, constructions and microelectronics [5, 6, 7]. Consequently, the usage of these industrial materials is expected to increase in developing countries such and Myanmar and others Asia countries. Proper usage of these industrial materials depends on basic research data as well as proper education and knowledge sharing to achieve sustainable and safe industrial development inside Asia as well as global. From this point of view, surface properties of these industrial materials such as elemental states, binding energy and chemical composition become important in international standards. In this work, the development of spectral data base for (T-M-C) by X-ray photoelectron spectroscopy (XPS) will be presented. The data base spectra were accumulated according to ISO standards measurement procedures and techniques such as binding energy calibration and sample cleaning. Among (T-M-C) materials, VC, HfC and Cr3C2 are of high possibility for many applications in future industry due to their unique physical properties like hardness, high melting temperature and high thermal expansion coefficient [8,9,10]. Spectral analysis for database of these materials will be discuss in detail.

2. Experimental

The experiments were carried out with ULVAC PHI 5500 XPS system equipped with a hemispherical energy analyzer (PHI model 10-360) along with monochromatic Al-kα (hν =1486.6eV) X-ray source, a differentially pumped ion gun (04-303), electron flood gun (04-090). Base pressure of ultrahigh vacuum system during analysis was 2.8x 10-8 Pa. Pass energy 11.75eV

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and energy step 0.1eV were used throughout the measurements. A vanadium carbides single crystal VC (100) (Ma Teck GmbH) with 5mmφx 1mmt in size and a VC sintered sheet (99% purity, Furuuchi Chemical Co.) with 10 x 10 x 1mmt in size are used in the experiments. The sample cleaning was done with Ar+ ion gun sputtering. The sputtering conditions used were emission current 25mA at 1kV, raster size 2 x 2mm and Ar pressure at 20 x 10-3 Pa. An electron flood gun and a thin Al foil were used for compensation of charge effect. The energy calibration of an instrument was carried out before measurements started using the standard metals Au, Ag and Cu. The details of binding energy calibrations steps were as follows. Standard metal Au, Ag and Cu samples (purity 99.99%, Furuuchi Chemical Co.) with 7 x 7 x 1mmt in size were prepared in one sample holder in order to get accuracy as much as possible. Sputter cleaning was done before measurements and confirmed to be clean by survey spectra in which the heights of the C1s and O1s peaks were less than 1% of the main metal peaks. Photoelectrons peaks of Au4f7/2, Cu2p3/2 and Ag3d5/2 were analyzed accordingly and measurements were repeated for 7 times. From the measurements results of Au4f7/2, Cu2p3/2 and Ag3d5/2 binding energy peaks the instrument linearity, uncertainty at 95% confidence and correction factors A (energy scaling errors) and B (zero offset errors) were calculated. The detail of analysis procedures and the calibrations procedures were carried out according to ISO15472 [11, 12]. The calculated energy calibration value ΔEcorrection was found to be -0.6796eV. The results of calibration and value of photoelectron peaks after energy calibration were shown in Table 1. In the present work, aiming for high accuracy, ΔEcorrection value was used in binding energy corrections for obtained data.

3. Results and Discussion

In the figures (a) and (b) and (c) represent spectra of V2p, C1s and O1s peaks respectively while [I] and [II] represent the spectra taken before and after sputtering. Fig. 1 and Fig. 2 compared the typical XPS spectra of V2p3/2, V2p1/2, C1s and O1s obtained from VC (100) and VC sintered samples.

Fig. 1 (a) showed the V2p photoelectron peaks of VC (100) sample before and after sputtering. V2p3/2 and V2p1/2 photoelectron peaks at 513.32eV - 520.8eV and 516.786eV - 523.95eV were assumed as metallic and oxide peak. It is likely that the sample surface was composed with both vanadium carbide and vanadium oxide at as-received condition. M.D Antonik etc. [13] have carried out the oxidation behaviour of VC (100) and similar XPS spectra were obtained. After sputtering for 2 minutes, well-resolved photoelectron peaks appeared. The energy separation between two photoelectron peaks 513.02eV and 520.68eV was 7.68eV, which confirmed the spin orbit component of V2p3/2 and V2p1/2. The reference value of energy separation was 7.7eV for vanadium metallic peak [14]. The third peak appeared at 530.8eV was assigned as O1s peak. In (b), C1s spectra before sputtering showed three photoelectron peaks at 282.41eV, 284.42eV and 288.21eV, respectively. A similar XPS spectrum of C1s in VC sample was reported by J.G. Choi etc. [15]. In this report three carbon peaks were assigned as hydrocarbons adsorbates on surface (Ca) such as CO, CO2 and at 285.6eV, free carbon contaminated on carbide surface (Cf) at 284.3eV and carbidic carbon (Cc) at 282.4eV from vanadium carbide lattice. Photoelectron peaks at 282.41eV and 284.42eV obtained in present work were well-matched with ref. [15], assuming carbidic carbon (Cc) from vanadium carbide lattice and free carbon (Cf) which was graphite-like carbon contaminated on carbide surface. The third peak 288.21eV has different binding energy compared to ref [15]. However, at (b), after 2 minutes sputtering the spectra showed 288.21eV peak disappeared completely suggested that this third peak was surface adsorbates which is similar to ref. [15]. In (II), only 282.41eV peak remained with well-resolved and other two peaks disappeared. At this state, the ratio of V2p3/2 and carbidic carbon (Cc) intensity (in peak area) was 1.27 ≅ 1.3. This suggested photoelectron ejected at

513.2eV was ascribed to V1.3+. This value indicated that the absolute magnitude of charge transfer from V 3d states to C2p states is 1.3 electrons per vanadium in vanadium carbides [15]. In (c), O1s spectra before sputtering showed the convoluted photoelectrons peak. The deconvoluted peaks at 531.49eV and 529.92eV were assigned as chemisorbed or lattice oxygen dissolved into the vanadium carbide (Oa) and oxygen in vanadium oxide (Oo) such as VO2 and V2O5 [15]. After 2minutes sputtering the intensity of O1s peak decreased. Photoelectron peak at 531.49eV and 529.92eV was likely convoluted and appeared at 530.91eV. This result suggested that after 2 minutes sputtering the oxide peaks decreased distinctly and appeared at 530.91eV.

Although, there was small amount of oxide remained the sputtering was stopped to prevent the change in surface morphology of sample.

VC Sintered sample

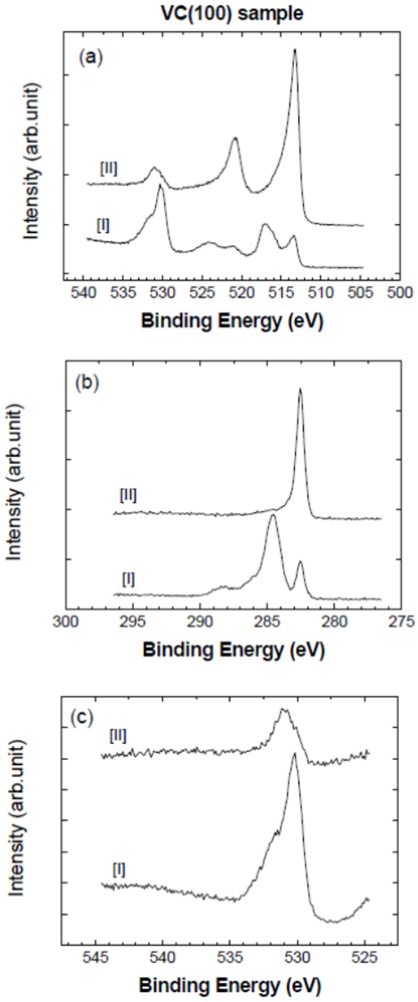

Figure1. Typical XPS spectrum of V2p3/2, C1s and O1s core levels for VC (100) sample. (a) V2p3/2 spectra. (b) C1s and (c) O1s. In the figure [I] represent spectra taken before sputtering and [II] represent spectra taken after 2 minutes sputtering.

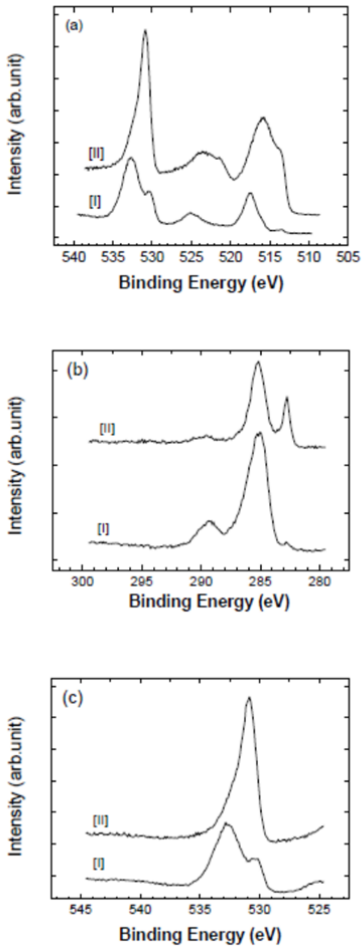

Figure2. Typical XPS spectrum of V2p3/2, C1s and O1s core levels for VC sintered sample. (a) V2p3/2 spectra. (b) C1s and (c) O1s. In the figure [I] represent spectra taken before sputtering and [II] represent spectra taken after 2 minutes sputtering.

In Fig. 2, XPS spectra obtained from VC sintered sample were analyzed. In (a), photoelectron peaks at 517.28eV-524.83eV has energy separation 7.55eV suggested the V2O5 oxide state. Different from VC (100) sample, there was no vanadium carbide related peaks in the spectra. It is likely that the sample surface was strongly covered with oxide at this state. After 20 minutes sputter cleaning convoluted peaks appeared rather similar to spectra of VC (100) sample before sputtering. Five deconvoluted peaks of vanadium oxide and vanadium carbide were obtained and summarized in Table 2. It is likely that during sputtering vanadium oxide changed to vanadium carbides. At the same time the oxygen dissolved in the vanadium lattice increased due to increase of high surface area vanadium carbide. In (b), two photoelectron peaks appeared in C1s spectra after 20 minutes sputtering and the third peak assigned as surface adsorbates was almost disappeared. Different from single crystal, the free carbon contaminated with carbides at 285.05eV remained together with vanadium carbide photoelectron peak at 282.69eV. In (c), O1s peak appeared at (a) were taken as narrow scan again. It was appeared that O1s photoelectron peak consist two deconvoluted peaks before sputtering. After 20 minutes sputtering photoelectron peak at 531.92V appeared assuming it was originated from lattice oxygen dissolved in vanadium carbide. Fig. 3 showed the deconvolution of V2p peaks for VC (100) and VC sintered sample before and after sputtering. Multipak (Ver.5)

PHI software with Shirley background subtraction method was used in data analysis. The photoelectron peaks obtained before and after sputtering were compared. All the other obtained results were also done by Multipak software and the binding energy of deconvoluted peaks were summarized in Table 2. The results showed 513.32eV-520.80eV attributed to carbide and 516.78eV-523.95eV attributed to oxide. The oxide shifted to carbide during sputtering resulted as (516.76→514.08→513.02eV). In the sintered sample oxide phase changed to carbide after sputtering at (517.28→515.65→513.52eV)

Table1. Calculated binding energy calibration values from data obtained by 7 times repeated measurements of standard metals Au, Ag and Cu.

Table 1. Caluculated binding energy calibration with uncertainty at 95% confidence from mesured Au, Ag and Cu for 7times repeatations.

Peak	Measured mean value	Corrected mean value	ISO ref.[11]	Hand Book ref.[14]	Repeatabilty standard deviation SD
	(eV)	(eV)	(eV)	(eV)	(eV)
$Au4f_{7/2}$	84.64	83.96	83.96	83.8	±0.021
$Ag3d_{5/2}$	368.77	368.09	368.21	367.9	±0.007
$Cu2p_{3/2}$	932.94	932.26	932.62	932.4	±0.013
Measurement of scale of non-linearity ε		0.0006			
Uncertainty of calibration of measurements U_{95}		0.055			
Calculated energy calibration value $\Delta E_{correction}$		−0.6796			

Table2. XPS binding energies (eV) of V2p3/2, Hf4f7/2, Cr2p3/2, C1s and O1s core levels for VC (100) single crystal, VC, HfC and Cr_3C_2 sintered samples.

Peak	VC(100)		$VC_{sintered}$		$HfC_{sintered}$		$C_{r3}C_{2\ sintered}$	
	Sputter Time 0 minute	Sputter Time 2 minutes	Sputter Time 0 minute	Sputter Time 20 minutes	Sputter Time 0 minute	Sputter Time 20 minutes	Sputter Time 0 minute	Sputter Time 20 minutes
M–C	513.32	513.02	513.52	513.25	14.93	14.99	574.42	574.51
	515.09	514.08	515.65	515.55	16.44	16.7	576.66	575.82
	516.78	516.76	517.28	518.25	17.66	18.9		577.55
	520.8	520.68	521.21	521.17	19.3		583.53	583.77
	523.95	523.76	524.11	523.87			586.48	586.8
C1s	282.41	282.4	282.67	282.69	281.9	282.26	283.09	283.12
	284.42	283.43	284.77	285.05	285.8	285.3	285.24	284.33
	288.21		289.21	289.5	289.32		299.04	
O1s	529.92	530.8	530	530.4	530.58	531.21	530.37	530.52
	531.49	530.91	532.42	531.92	532.43	532.75	532.29	532.13

Table 2 summarized the binding energy (eV) of deconvoluted core-level photoelectron peaks of V2p, Hf4f, Cr2p, C1s and O1s of all samples. In the present work, energy calibrated correction factor ΔEcorrection was applied to measured data for accuracy and binding energy for core-level photoelectron peaks were well matched with reference data.

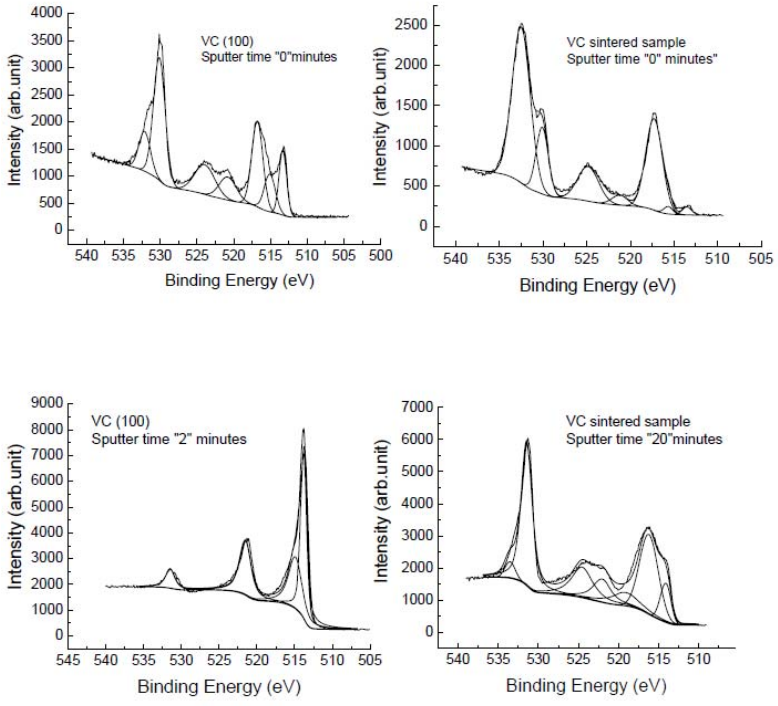

Figure3. XPS spectra of deconvoluted peaks for V2p before sputtering. (a) VC (100) sample and (b) VC sintered sample.

Fig. 4 showed the relative intensity ratio (in atomic concentration) Carbon/Metal (C/M) for VC (100) as well as VC, HfC and Cr3C2 sintered samples. In VC sintered sample C/M value reached to 0.67 after 10 minutes sputter time and become constant. In case of VC (100) sample the same value 0.67 was obtained after 2 minutes sputter time. For HfC this value was 0.24 and for Cr3C2 it was found to be 1. Compare to VC and HfC, Cr3C2 showed different situation which has large carbon concentration at 5minutes sputter time. The reason for this was not clear at the moment. One possible reason might be as received condition of sample was different from other.

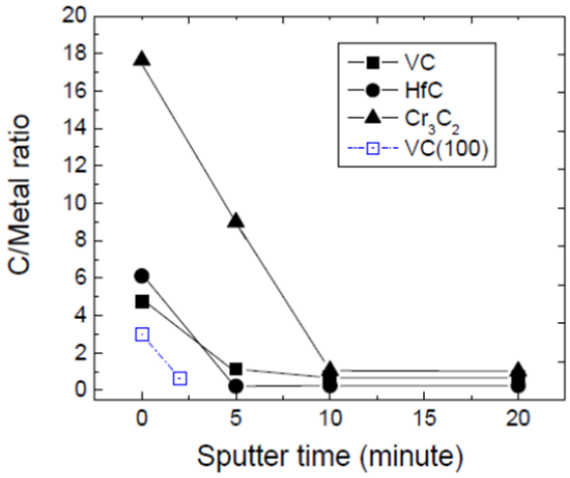

Figure4. Relative intensity ratio

Fig. 4 Relative intensity ratio (atomic concentration) of carbon/Metal (C/M) for VC (100) sample as well as VC. HfC and Cr3C2 sintered However, after sputter for 10minutes the C/M element ratio was quite stable and constant. These results showed the effect of remained species on surface such as oxide were not observed in present work.

4. Conclusion

XPS analysis of transition metal carbides (T-M-C) samples, VC (100) and VC sintered samples was carried out and surface properties changes due to the Ar^+ion sputtering was compared with HfC and Cr3C2. Aiming for the practical usage of spectra data of T-M-C, the surface cleaning was done using Ar^+ion sputtering attached in instruments and no other cleaning process such as chemical treatment or sample heating were carried out. In VC (100) sample well-resolved V2p and C1s photoelectron peaks were obtained after 2 minutes while for sintered samples the surface composition changes until 10minutes sputtering. Ar^+ion sputtering was carried out to take into an account that surface morphology has less effected. Although, there was some amount of surface contaminated O remained relative intensity ratio (in atomic concentration) Carbon/Metal (C/M) showed the constant nature and the effect of remained surface contaminated species such as oxide were not observed. During sputtering, oxides transformed to carbide and charge transfer occurred at early stage of sputter time. The carbidic carbon peaks observed were 282.4±0.3eV, 282.69±0.2eV, 292.26±0.4eV and 283.12±0.2eV for single crystal VC (100), sintered sample VC, HfC and Cr3C2, respectively. The instrument

binding energy calibration was carried out according to ISO standard and calculated $\Delta E_{correction}$ value was -0.6796eV. This correction value was applied to measured data and ejected photoelectron peaks were well matched with reference data. The Ar^+ ion sputtering method can be use as practical tools as simple method for sample cleaning in analysis of XPS.

Acknowledgements

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Distribution of Water Resources and their Management for Southern Shan State

Saw Yu May[1]

ABSTRACT

Water resource is the most basic needs for human life. The essential and important of water resources cannot be fully explained. Nowadays, water resources are major stressed around the world due to many reasons. Myanmar is one of the rich water resources countries in Southeast Asian Countries which will support to the socioeconomic development potential. For sustainable development, the existing distribution of water resources of an area is very important. Therefore, the main questions of this study to be addressed on how many types of water resources can be found and how it is distributed in the study area and what are the potential water resources of this region. The major aim of this study is to provide the baseline data of water resources in Southern Shan State and that will be effective management depending upon the types of water resources and its distribution of Southern Shan State. To fulfill the aim of this study, hydrological and meteorological analysis and GIS technique will be applied. Form the results of this study, the regional development can be implemented based upon the natural water resources.

Keywords: water resources, southern Shan State, water management

1. Introduction

Water resources are natural resources of water that are potentially useful. Water information is a fundamental to natural and local economic well-being, protection of life and property, and effective management of the Nation's water resources (Source: USGS). Myanmar is one the rich water resources countries in Southeast Asian Countries which will support to the socioeconomic development potential. Generally, there are three main types of water resources in Southern Shan State namely, rain water, ground water like the other part of Myanmar. For sustainable development, the fundamental facts about water resources of the area are very important. Therefore, it is necessary and important to study about the water resources and its management in Southern Shan State.

Research Questions and Aim and Objectives of the Study

Therefore, the main questions of this study to be addressed on how many types of water resources can be found and how it is distributed in the study area and what are the potential water resources of this region.

The major aim of the study is to provide the baseline data of water resources in Southern Shan State and that will be effective management depending upon the types of water resources and its distribution of Southern Shan State. The objectives of this study are to observe the current water resources and its distribution of Southern Shan State, to suggest the management of water resources within Southern Shan State.

Research Methodology

Secondary data sources which related with water resources from different Administrative departments like Meteorology and Hydrological Department, Department of Agriculture,

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Directorate of Water Resources and Improvement of River System Department, Agriculture and Irrigation Department and General Administrative Department for each township were collected. In order to obtain the distribution of water resources in Southern Shan State, GIS technique assist to generate mapping within this area depend on the availability data sources. Total coverage distribution patterns of rain water resources in Southern Shan State, the interpolation method was applied for the aid of average annual rainfall data which was acquired from the Meteorology and Hydrology Department, Taunggyi, Shan State and Department of Agriculture each township level because there are only five Meteorology and Hydrology Department within Southern Shan State in the towns of Taunggyi, Heho, Pinlaung, Namsang and Loilam. For these five towns, efficient water of these areas could be portrayed.

Study Area: Southern Shan State

Shan State, the biggest area of Myanmar, is situated in the eastern most part of Myanmar which has an area of about 155,800 square kilometers covering almost a quarter of the total area of Myanmar. For absolute location, it is located Latitude between 19° 18′ and 24° 05′ North and Longitude between 96° 13′ and 101° 11′ East. Generally, Shan State can be divided into three parts administratively: Southern Shan State, Northern Shan State and Eastern Shan State. In Southern Shan State, there are 3 districts which comprising 21 townships.

DISCUSSIONS AND FINDINGS

Regarding with water resources data in Southern Shan State, it can be divided into three main water resource types namely in Southern Shan State: rain water resource, surface water resource and ground water resource.

2. Rainwater Resource Southern Shan State

In southern Shan State, rainwater is one of the valuable water resources in many places of Shan State. According to the rainfall and rainy day data from meteorological and Hydrology Department in Taunggyi and Department of Agriculture in District level, the average total annual rainfall and rainy day can be generally concluded. Based on these data, the average total annual rainfall and rainy day are varied from township to township in Southern Shan State where the average total annual rainfall is significantly varied between about 600 millimeter (23.6 inches) and over 2300 millimeter (90.5 inches) from area to area due to its location and geographical feature.

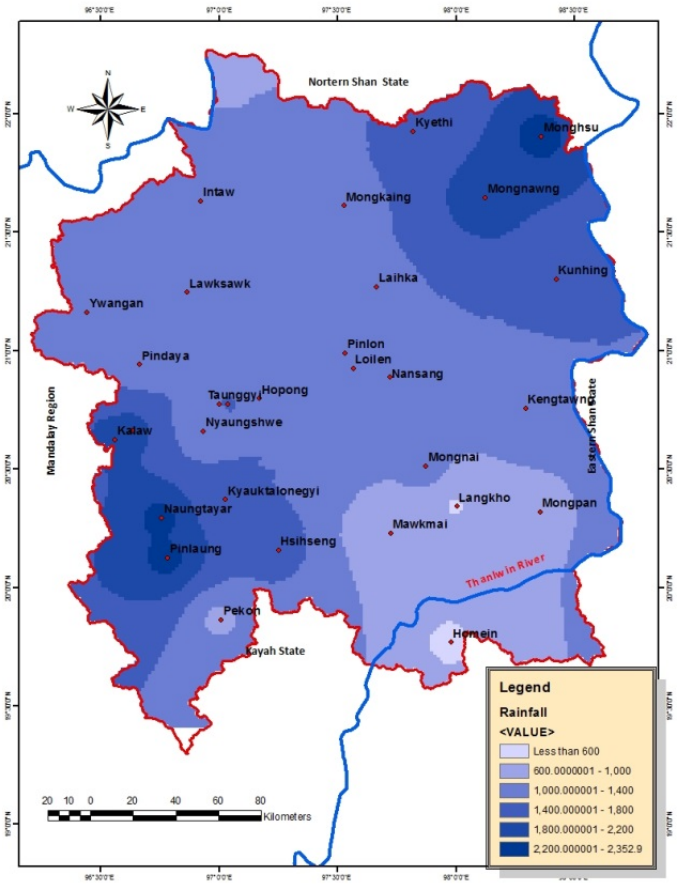

Figure1. Average Annual Rainfall Distribution Patterns of Southern Shan State

Source: Meteorology and Hydrology Department, Taunggyi and Department of Agriculture, each District in Southern Shan State

While the highest the average total annual rainfall in Southern Shan State is in Mongshu Township which amounted with 2353.6 millimeters (92.7 inches) and in Pinlaung Township which accounting for 2343.5 mm (92.3 inches) due to its location and physical feature, the lowest amount of the average total annual rainfall was found in Mawkmai Township, Langhko Township and Pekhon Township with the average annual total amount of rainfall is about 620.3 millimeters (24.4 inches), 859.3 millimeters (35.2 inches) and 897.4 millimeter (35.3 inches), respectively.

Surface Water Resource in Southern Shan State

Regarding with surface water resources in Southern Shan State, rivers, ponds, dams or reservoirs and hand dug well contributes to the sources for water supply in this type. All these form of surface water are contributed as water resources and water supply of Southern Shan State.

The main river in Southern Shan State is Thanlwin (formally known as Salween River) and its tributaries. It flows from north to south in the eastern part of Southern Shan State and form as a natural boundary with Eastern Shan State. The whole Thanlwin River Watershed is about 158,000 square kilometer (Source: INWRC). In the eastern, northern and southern part of

Southern Shan State are part of Thanlwin River watershed and all the tributaries and sub-tributaries are flowing into it.

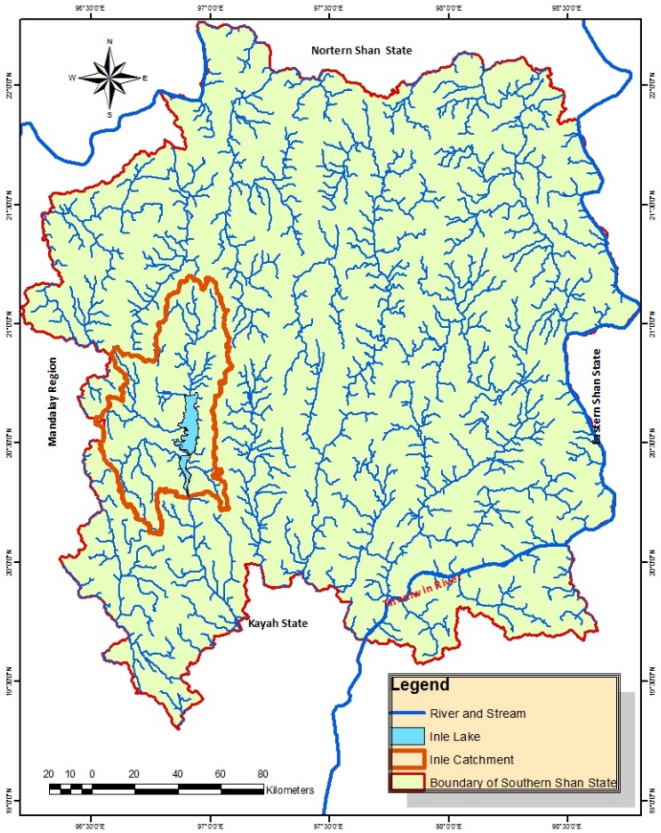

Figure2. Distribution of River, Stream and lake water Resource that use for fresh water resources in Southern Shan State Source: USGS, MIMU (Myanmar Information Management Unit)

Another surface water resource in Southern Shan State is lake or pond water resources. There are many natural ponds and artificial ponds for agriculture and water supply purposes in Southern Shan State. Among the natural lake, Inle Lake, the second largest lake of Myanmar, is situated in the Nyaung Shwe Basin at 2900 feet above mean sea level, is popular for it natural scenic value and unique culture. It was designated as ASEAN Heritage Park in 2003, IBA-(Important Bird and Biodiversity Area) in 2004, UNESCO Biosphere Reserve in 2015 and Wetland of International Importance Ramsar Site in 2018.Dam or reservior is one of the irrigation water management activities which allocation of water for agriculture.The water are distributed from the dams via gravity and through pumping stations. According to the data obtained from Ministry of Irrigation and Agriculture, there are 341 dams/reserviors in 2018 within Southern Shan State.

3. Ground Water Resources in Southern Shan State

Ground water resource is one of the important fresh water sources of the earth. Being as a large Karst land area, some of the surface area like streams disappeared or dissolved into the ground (sinkholes) for a long distance by forming underground water drainage system and appear again from cracking rock or ground which is named springs (is called Yae-htwet by local people).

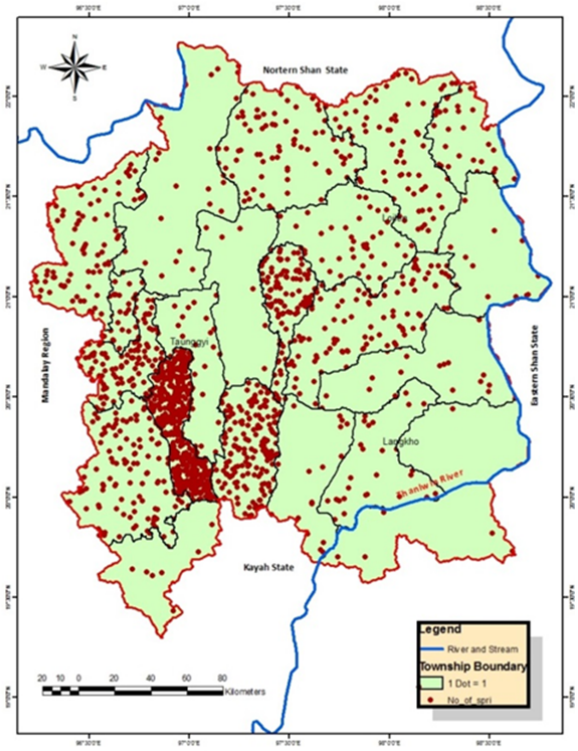

Figure 3. Distributions of Spring Water Resources by District in Southern Shan State

Source: Directorate Water Resources and Improvement of River System Department, Taunggyi

There are 1521 springs in Southern Shan State, among them 1206 springs which represent 79 percent of springs can be located within Taunggyi District; about 216 springs which is about 7 percent exist in Loilen District and only 99 springs which estimate only 4 percent fall within Langkho District. Among the townships, Nyaung Shwe Township has the highest number of springs which accounted for 467 springs. See in Figure 3. Apart from the springs, other ground water sources are dug well and tube well those contribute to the water resources of Southern Shan State

4. Conclusion

As result of the assessment on the three water resources of Southern Shan State, water Management should implement based on the existing water resources in Southern Shan State.

Taunggyi District has moderate amount of rainfall except Pinlaung Township which has above moderate amount of rainfall. Similarly, Loilen District has moderate amount of rainfall apart from Mongshu where has above moderate amount, it is significant that Langkho District has under the moderate amount of rainfall. Therefore, rain water harvesting, conservation and systematic management take into account for rain water resource within Taunggyi and Loilen Districts and support for local water supply as a valuable resources both urban and rural area.

About 95% of river drainage system in Southern Shan State is Thanlwin River watershed area. It should be a valuable water resource for Langkho District because there is little rainfall.

There are different sizes of dams/reservoirs across the whole Southern Shan State, some are owned by government and some are private. But it is necessary to study water resources of each and every dam and how to use sustainable agriculture management.

Spring water is also very important water resources for Southern Shan State. But currently, many springs are vulnerable due to land use changes which have to urgently conserve. Ground water is the most using water resources in Southern Shan State which represent about 64.4% of the whole water resources although there have other form of resources in this area.

Management, preparation and systematic plan are necessary impacts of climate change, land use change and natural disaster to water resource for sustainability.

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Synthesis and Characterization of Rice Husk Derived Bioactive Glass Ceramic

Min Maung Maung[1], Myat Moe Aye[2], Aung Min[3]

Abstract:

The raw material, rice husk was going through thermal treatment in order to produce white ash powder and milled into nano-sized powder. The ash samples were characterized by X-ray diffraction (XRD), scanning electron microscopy technique (SEM), Fourier transform infrared spectroscopy (FTIR) and Energy-dispersive X-ray spectroscopy (EDX). Bioactive glass-ceramics was synthesized through sol–gel route using these rice husk ash powder as silica source. Scanning electron microscopy - energy dispersive spectroscopy (SEM-EDX) and X-ray diffraction were used to monitor the surface deposition on glass-ceramics during incubation. Furthermore, UV-Vis absorbance and transmittance measurements were carried out to complete the characterization. The results showed that the glass synthesized using silica from rice husk exhibited a good bioactivity with a formation of carbonated hydroxy apatite phase in 3 days of incubation.

Keywords: Rice husk ash (RHA), bioactive glass, carbonated hydroxy apatite, sol-gelroute.

1. Introduction

One of the major food crops in the world is the production of rice and which generates rice husks (RHs). It is one of the major sources of wastes of the world. When RH is combusted in air, the combustibles can be mostly burned off and some solid products are left, which are named rice husk ash (RHA). The predominant component of RHA is silica. The abundant, cheap, regenerable RHs naturally have high contents of silica and the silica has a high reactivity. Thus, how to extract highly pure silica from RHs, and make an efficient use of it, has recently received more and more attention.

Silica in crystalline form induces adverse biological effects, while amorphous silica is known to be bioactive, biocompatible, biodegradable, and non-toxic in living tissue, as well as in corresponding simulated physiological conditions. Nowadays, along with above stated amorphous silica-based materials, amorphous fumed and precipitated silicas are increasingly used in diagnostic and biomedical research.

Bioactive ceramics like, glass, glass-ceramics, hydroxyapatite (HAp), calcium phosphates, and calcium silicate have been used for practical clinical applications. They have attracted increasing attention as promising biomaterials for bone tissue engineering. Bioactive ceramics are used in the form of powder, densed ceramics, highly porous reticulated ceramics, mesoporous ceramics, etc. Dense and porous ceramics are fabricated through different methods like powder compaction, fiber bonding, solvent casting/particulate leaching, gas foaming, phase separation, replication technique, gelcasting, and sol-gel technique, etc. Silica-based glass and glass-ceramics are most bioactive among all bioceramics. Glass-ceramics have added advantage of better mechanical properties than glass.

In the present work, amorphous silica was extracted from rice husk collected near Mawlamyine township, Mon state, Myanmar. The structure characterization of rice husk ask was

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identified by X-ray diffraction (XRD), scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR) and the silica concentration was also be determined by Energy Dispersive X-ray (EDX) spectroscopy. Silica-based bioactive ceramics was prepared by Conventional sol-gel process and conventional pressing method. Their UV absorption and transmission measurement was done by the aid of UV-Vis spectrophotometer. The bioactive property was also being tested with In-vitro degradation method.

2. Materials and Method

2.1 Extraction of Silica from Rice husk

Rice husk (RH) was collected from a rice mill near Mawlamyine township, Mon state, Myanmar. The RH was separated from rice grain by air blowing and washed with tap water for several times till all the blackish impurity floating on water was completely removed. The rice husk was then dried at 110°C for 8 h. The dried husk was burning at 600°C, 700°C for 6 h for complete combustion by which all volatile material is removed and ash was obtained. The flow chart of extraction of silica was shown in figure 1.

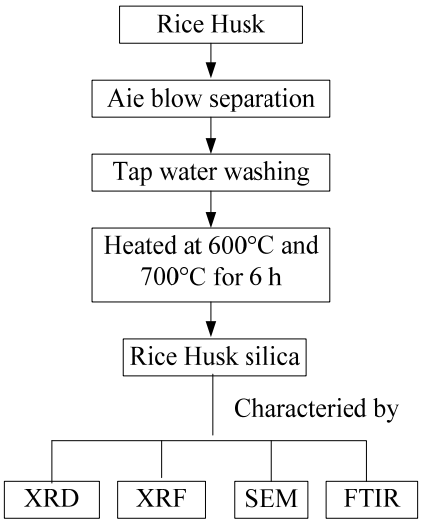

Figure 1 The flow chart of extraction of amorphous silica from rice husk.

2.2 Preparation of bioactive glass-ceramic powder from rice husk silica

RHS was taken as source of silica and sources of Na_2O and CaO were used as NaOH and $Ca(NO_3)_2.4H_2O$. In this process, 50mol% of SiO_2, 25mol% of Na_2O, 25mol% of CaO and 25mol% of P_2O_5 were used to obtain bioactive glass ceramic. Estimated NaOH was dissolved in 20 ml of deionized water and $Ca(NO_3)_2.4H_2O$ and 30 ml of deionized water in beaker separately to make clear solution. When NaOH solution was warmed, RHS and P_2O_5 were added into it in warm condition only and volume was made 60 ml by adding deionized water. The boiling was continued for 1 hr while volume was maintained upto 60 ml mark of beaker regularly at around time interval of 15 min. After 1 hr of boiling, RHS and P_2O_5 were dissolved completely. Then this solution was filtered and sodium silicate was obtained.

Estimated amount of $Ca(NO_3)_2.4H_2O$ solution was prepared by dissolving it in 30 ml of deionized water. Then 15 ml of HNO_3(conc) was added in this solution, which was kept in stirring condition, followed by addition of Sodium silicate solution drop wise very slowly. Initially, addition rate of Sodium silicate solution was 5 ml per min. At the end when 7-8 ml of Sodium silicate solution was left in beaker, rapid addition was made. Then it was left for 3 days at 70°C for ageing so that glass network formation is optimized. Then it was dried at 150°C for 2 days. The preparation step was specified in figure 2.

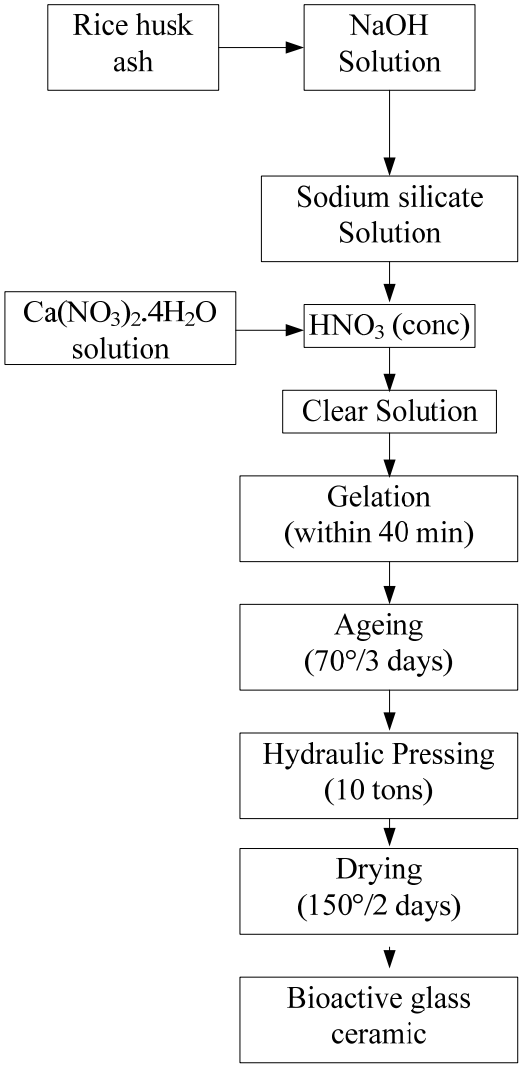

Figure2. The process of preparation of bioactive glass ceramic.

Figure 3. The photograph of silica based glass ceramic.

3. Results and Discussion

3.1 Characterization of rice husk silica

X-rays powder diffraction of the samples was carried out at room temperature using Rigaku using CuK_α (λ = 0.154 nm) radiation, with a diffraction angle between 20º and 70º to check the formation of the required product and structural related properties. **Figure 4 showed that the XRD pattern of** amorphous silica derived from rice husk. The spectrum appears as a broad band with the equivalent Bragg angle at 2θ = 22°, which indicates that the material is amorphous. The silica from the calcination of RH at 600 °C was amorphous. Calcination of RH at 700 °C was both amorphous and crystalline. **The percentage of amorphous silica was increased due to the wider of broadening of peak in rice husk ash.** For the non-crystallized ashes, the broad amorphous diffraction is decided by the short range order structure of the amorphous SiO_2.

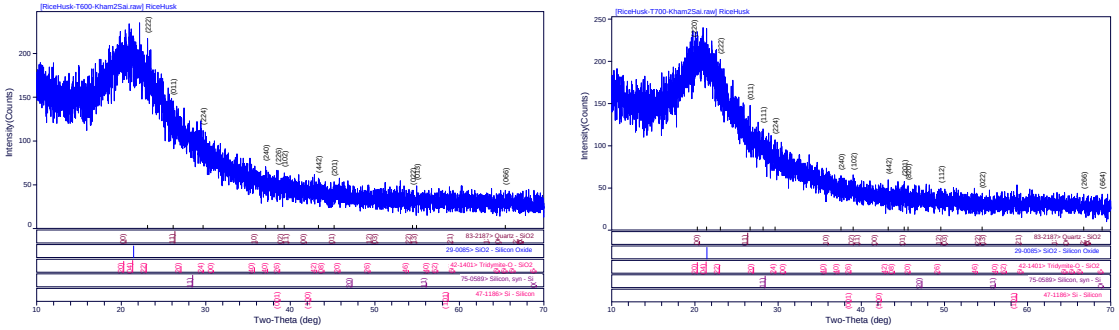

Figure 4. The XRD patterns of amorphous silica by annealing at 600℃ and 700℃ for 6 h

The element concentration of extracted Silica from Rice husk was determined by Energy dispersive X-ray Spectroscopy. The element contained in these samples was also listed in the table1.

samples	**SiO_2**	**Al_2O_3**	**Fe_2O_3**	**CaO**	**MgO**	**K_2O**	**MnO**	**TiO_2**
Raw rice husk	93.14	0.21	1.54	2.85	0.30	0.82	0.53	0.012
Rice husk ask annealed at 600°C	93.20	0.13	0.07	1.23	0.25	0.78	0.33	0.006
Rice husk ask annealed at 700°C	96.01	0.08	0.57	1.22	0.01	0.20	0.27	0.002

The surface morphology of the silica particles obtained after calcinations at 600°C and 700°C for 6 hours were shown in figure 6. The Scanning electron microscopy (SEM) micrographs illustrated that amorphous silica particles were obtained. The majority of primary SiO_2 particles had a uniform size varying from ~50 to ~ 70 nm in size. These primary particles showed a big tendency to aggregate and form larger particle clusters.

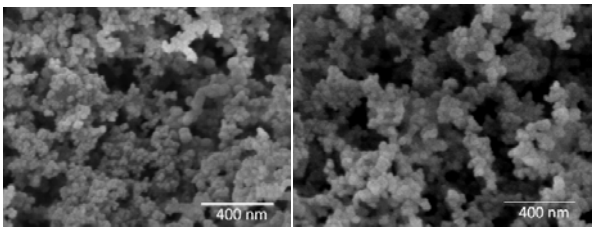

Figure5. The SEM micrographs of silica calcinated 600℃ and 700℃ for 6 h

The Fourier transform infrared (FT-IR) absorption spectrometry was performed and was taken in the IR reflection mode, in which the ash sample was directly pressed to form a pellet without addition of KBr. The broad band at 3410-3470 cm^{-1} was due to the stretching vibration of the O-H bond from the silanol groups (Si-OH) due to the adsorbed water molecules on the silica surface. The band at 2344 cm^{-1} was due to the carbonate which is absorbed in the sample from atmosphere during sample preparation. The band at 1090 cm^{-1} was due to the Si-O-Si asymmetric stretching vibration, while the band at 801 cm^{-1} had been assigned to the network Si-O-Si symmetric bond stretching vibration. The band at 470 cm^{-1} was associated with a network O-Si-O bond bending modes.

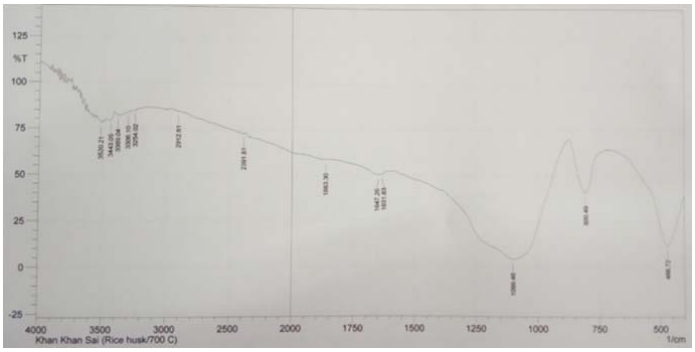

Figure6. The FTIR spectra of silica calcinated 700℃ for 6 h

3.2 Characterization of glass ceramic

The glass ceramic powders based on the amorphous silica extracted rice husk ash were sintered at 700°C, 800°C and 900 °C for 2 hr. X-ray diffraction pattern was confirmed that the presence of sodium calcium silicate on these ceramic samples and was shown in figure 7. The sample with sintering temperature at 700°C showed the presence of sodium calcium silicate with two different crystalline phases $Na_6Ca_3Si_6O_{18}$ and $Na_2Ca_2Si_2O_7$. The powder sintered at 800°C and 900°C also shown that the presence of $Na_6Ca_3Si_6O_{18}$ and $Na_2Ca_3Si_6O_{16}$ phases.

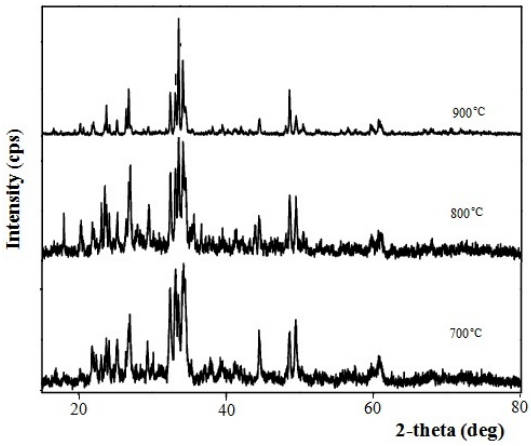

Figure 7. The XRD pattern of glass ceramic

Chemical analysis was performed with energy dispersive X-ray spectroscopy EDX in scanning electron microscopy SEM mode. The EDX analysis also showed the presence of C on its surface. Ca/P ratio had decreased to 2.37 from 2.97. The weight percent of Si and O had decreased due to formation of denser and spherical Carbonated Hydroxyapatite on the surface. The average grain size of this sample was found to be 1.06 μm.

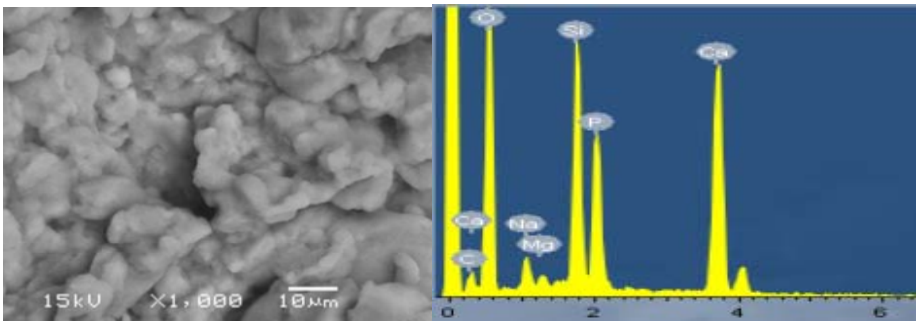

Figure 8. The SEM-EDX image of glass ceramic

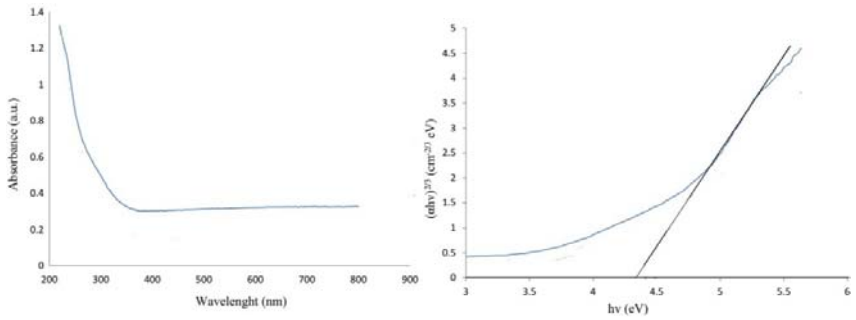

Figure 9. The UV absorbance and optical band gap of bioactive glass ceramic

The UV absorbance and plot of $(\alpha h\nu)^2$ versus photon energy ($h\nu$) of glass ceramic was shown in figure 9. In the direct transition, the absorption coefficient (α) related with the optical band gap (E_g) is given by, $\alpha h\nu = A(h\nu - E_g)^{1/2}$, where $h\nu$ is the photon energy and A is a constant for a direct transition. The energy gap E_g could be estimated from the intercept of $(\alpha h\nu)^2$ vs $h\nu$ for direct transitions. By extrapolating the linear portion of the energy axis at zero absorption gives the direct band gap of these materials. The energy gap of this sample was observed to be 4.3 eV.

In order to study the dissolution features of glass-ceramics, Tris buffer solutions was chosen because, Tris is the plain buffering agent used in most SBF preparations. Tris solutions do not contain ions and thus represent, for a bioactive material, maximum solubility and minimum reprecipitation activity. Pure Tris was dissolved in distilled water to obtain a concentration of 6.1 gpl. The solution pH was lowered to 8 by acidifying with a solution of 1 M HCl. In vitro bioactivity test showed the formation of apatite layer on silica specimen surface. Biodegradability test showed a substantial dissolution of silica network within six hours. The rate of silica network dissolution and apatite phase formation was dependent on impurity cation content in amorphous silica which was inherited from husk composition.

4. Conclusion

Highly bioactive glass ceramics have been synthesized using rice husk ash waste material as a source of silica. The amorphous and glassy nature was confirmed by XRD analysis. The shorter ageing time period and evaporation of unreacted phosphorus precursors was confirmed by EDX measurement. Sintered pellet with about 20% apparent porosity was tested for bioactivity and biodegradability. Crystalline combeite phase of the glass-ceramics was found to dissolve easily in Tris buffer solution. Carbonated hydroxyapatite was formed on the surface of the glass-ceramics within 3 days of incubation. It may be concluded that rice husk ash may be low costing raw material for the preparation of bioactive glass ceramic materials through simple sol–gel route.

Acknowledgements

I would like to express my profound thanks to Professor Dr Khin Khin Win, Head of Department of Physics, and Professor Dr Myo Lwin, Professor Dr Aye Aye Thant, Professor Dr Yin Maung Maung, Department of Physics, University of Yangon, for their kind permission to carry out this work, their encouragement and help during this work.

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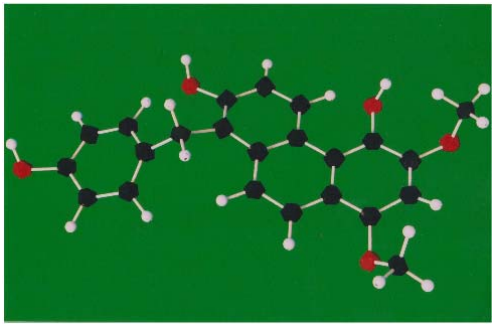
Antimicrobial Activity and Structure Elucidation of Isolated Phenanthrene Derivative Compound from the Root of *Atalantia monophylla* (Roxb.) DC. (Taw shauk)

Thinn Myat Nwe[1]

Abstract

In this research, one of the Myanmar indigenous medicinal plants, *Atalantia monophylla* (Roxb.) DC which is known as Taw shauk in Myanmar was selected. The preliminary phytochemical test for root of Taw shauk shows the presence of glycoside, phenolic, alkaloid, saponin, polyphenol, flavonoid, steroid and terpene groups. The antimicrobial activities of the ethyl acetate extract of crude sample responds high activity on *E. coli* and medium activities on *Staphylococcus aureus, Bacillus pumilus* and *Candida albicans*. Furthermore, phenanthrene derivative compound was isolated as pale yellow oily form by modern methods such as Thin Layer and Column Chromatography from the root of *Atalantia monophylla* (Roxb.) DC. The yield percent of this compound was found to be 0.06 % (1.4 mg) based upon the ethyl acetate crude extract. This compound gives positive test for phenol. Antimicrobial activities of this isolated compound respond medium activities on all selected organisms except *Bacillus subtilis*. In addition, the molecular formula of isolated compound is $C_{23}H_{20}O_5$. The hydrogen deficiency index of this isolated compound is 14. The planar structure of isolated compound is as follow



Keywords: Tawshauk,phenanthrene,$C_{23}H_{20}O_5$, antimicrobial activity

1. Introduction

Traditional Systems of medicines always played important role in meeting the global health care needs. They are continuing to do so at present and shall play major role in future also. The system of medicines which are considered to be Indian in origin or the systems of medicine, which have come to India from outside and got assimilated in to Indian culture are known as Indian Systems of Medicine (Prasad, 2002). India has the unique distinction of having six recognized systems of medicine in this category. They are Ayurveda, Siddha, Unani and Yoga, Naturopathy and Homoeopathy. Though Homoeopathy came to India in 18th Century, it completely assimilated in to the Indian culture and got enriched like any other traditional system hence it is considered as part of Indian Systems of Medicine (Prasad, 2002). The therapeutic properties of medicinal plants are conditioned by the presence in their organs of active substances, such as alkaloids, flavonoids, glycosides, vitamins, tannins, and coumarin compounds, which physiologically affect the bodies of humans and animals or which are biologically active in relation to the causative agents of various diseases (Yirga, G., Teferi, M.

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and Kasaye, M. 2011). Also in Myanmar, medicinal plants are abundant. Most of the people used the traditional medicinal plants for the treatment of diseases and to relief pain. The study of traditional indigenous medicinal plants and their usage in therapy play a very important role (Lashari, M.S. 1984). Traditional medicinal practices are an important parts of the primary care delivery system in most of the developing world. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenances of good health, has been widely observed. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs from these plants as well as from traditionally used rural herbal medicines (A Cheikyoussef, *et al*, 2011).

2. Methods

Apparatus and Materials

The sophisticated instruments which are used in the isolation and structure elucidation of pure compound are presented as follows.

1. UV-lamp (Lambda-40, Perkin-Elmer Co., England)
2. FT-IR Spectrophotometer (Shimadzu, Japan)
3. ^{1}H NMR Spectrophotometer (500 MHz)
4. ^{13}C Spectrophotometer (125 MHz)
5. EI-Mass Spectrophotometer
6. Electric balance (Shimadzu, Japan)

Commercial grade reagents and solvents were used after distillation. Silica gel (Merk Co. Inc, Kiesel gel 70-230 Mesh ASTM) was used for column chromatography. Iodine vapor and UV detector were used for visualizing the compound situated on TLC plates.

Sample Collection

Plant materials were collected from Wachet Village, Sagaing Region. The collected sample, the root of Taw shauk was cut into small pieces and was dried in the shade. Then, the raw materials were kept in the glass bottle with stopper and used throughout the experiment.

Aim and Scope of the Present Research Work

The aim of the present research work is the structure elucidation of bioactive pure organic compound isolated from the root of *Atalantia monophylla* (Roxb.) DC.

The scope of this research work is outlined as follows;

(i) To study the antimicrobial activities of crude extracts of medicinal plant, Taw shauk

(ii) To isolate a bioactive organic compound from Taw shauk by utilizing solvent extraction method followed by column and thin layer chromatographic separation methods.

(iii) To elucidate the structure of isolated compound by using sophisticated spectroscopic methods.

Botanical Description

Family	:	Rutaceae
Botanical name	:	*Atalantia monophylla* (Roxb.) DC.
Local name	:	Taw shauk
Parts used	:	root
Medicinal Uses	:	Painful joints and rheumatism

Figure1. The Plant and Root of Atalantia monophylla (Roxb.) DC.

Extraction and Isolation of Isolated Compound from the root of *Atalantia monophylla* (Roxb.) DC.

The air dried sample of the root of Taw shauk (570 g) was percolated with 95 % ethanol (1.35 L) for about 2 months. Then the percolated solution was filtered and evaporated to be concentrated. The ethanol extract was extracted with ethylacetate (400ml). The EtOAc crude extract (2.3 g) was chromatographed by silica gel column by using n-hexane and EtOAc, with various solvent ratios from nonpolar to polar. Totally 213 fractions were obtained. Each fraction was checked by TLC. The same Rf value fractions were combined. Totally, 18 combined fractions were obtained. Among them, fraction IX found as main fraction. The pure pale yellow oily form (0.0014g) was obtained and its Rf value is 0.37 (nhexane and ethyl acetate, 1 : 1). The yield percent of this pure compound was found to be 0.06% based upon the ethylacetate crude extract.

3. Results and Discussion

Preliminary Phytochemical Screening of the Root of *Atalantia monophylla* (Roxb.) DC. (4, 8)

Phytochemical Screening was carried out by general methods to indicate the presence of general classes of phytoconstituents. In accordance with phytochemical tests, it contains alkaloid, flavonoid, glycoside, steroid, terpene, polyphenol, phenolic and saponin. The results are shown in Table (1).

Table (1) Preliminary Phytochemical Screening of the Root of Taw shauk

No	Constituents	Reagents used	Observation	Result
1	Alkaloid	Dragendroff' reagent	orange ppt	+
2	Flavonoid	EtOH, Conc: HCl + Mg turnning	Red	+
3	Glycoside	10% Lead acetate solution	no change	-
4	Steroid	Petether, Acetic anhydride, conc S/A	Green	+
5	Terpene	$CHCl_3$ + Acetic anhydride + Conc:H_2SO_4	no change	-
6	Poly phenol	1% $FeCl_3$ + 1% $K_3Fe(CN)_6$ solution	Bluish green	+
7	Phenolic	10% $FeCl_3$ solution	Bluish green	+
8	Saponin	Distilled water	Frothing	+

(+) sign indicates the presence of the constituent (-) sign indicates the absence of the constituent

Antimicrobial Activities of the Root of *Atalantia monophylla* (Roxb.) DC.

Table (2) Antimicrobial Activities of the Root of Taw shauk

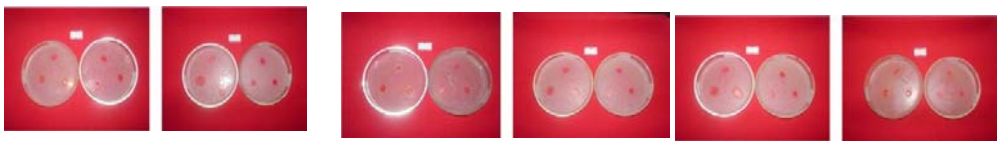
Solvents	Types of organisms					
	I	II	III	IV	V	VI
EtOH	-	18 mm (+ +)	-	15 mm (+ +)	17 mm (+ +)	-
EtOAc	-	17 mm (+ +)	-	15 mm (+ +)	17 mm (+ +)	20 mm (+++)

Organisms 7 mm-11 mm (+) - low activity, 12 mm-16 mm (++) - medium activity, 17 mm above(+ + +) - high activity

I = *Bacillus subtilis,* II = *Staphylococcus aureus*, III = *Pseudomonous aeruginosa,*

IV = *Bacillus pulmilus*, V = *Candida albican*, VI = *E coli*

According to this table, ethyl acetate extract of Taw-shauk responds high activity on *E. coli* and medium activities on *Staphylococcus aureus, Bacillus pumilus* and *Candida albicans.* And then EtOH extract gives medium activities on *Staphylococcus aureus, Bacillus pumilus* and *Candida albicans*. n-Hexane extract gives no activity on any of the six organisms.



Bacillus subtilis *Staphylococcus aureus* *Pseudomonous* *Bacillus pulmilus* *Candida albican* *E.* coli

Figure 2. Antimicrobial Activities of the Root of Taw shauk

Antimicrobial Activities of Isolated Compound

The antimicrobial activities of compound were tested by using Agar-well diffusion method. These results were shown in Table (3). These results inform that ethanol extract of isolated compound responds medium activities on *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *E .coli.*

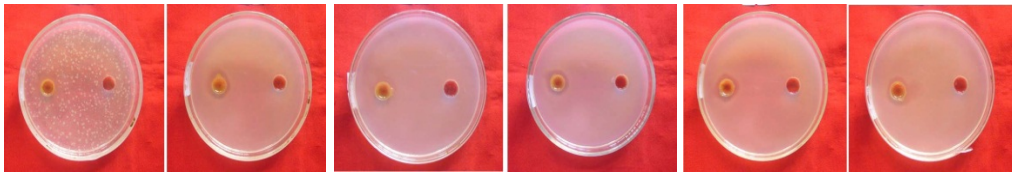
Table (3) Antimicrobial Activities of Isolated Compound

Plant Sample	**Solvent**	**Organisms**					
		I	**II**	**III**	**IV**	**V**	**VI**
Isolated Compound	EtOH	13 mm (+)	18 mm (++)	17 mm (++)	16 mm (++)	15 mm (++)	16 mm (++)
Control	EtOH	-	-	-	-	-	-

Organisms 7 mm-11 mm (+) - low activity, 12 mm-16 mm (++) - medium activity, 17 mm above(+ + +) - high activity

I = *Bacillus subtilis,* II = *Staphylococcus aureus*, III = *Pseudomonous aeruginosa,*

IV = *Bacillus pulmilus*, V = *Candida albican* VI = *E coli*



Bacillus subtilis *Staphylococcus aureus* *Pseudomonous* *Bacillus pulmilus* *Candida albican* *E.* coli

Figure3. Antimicrobial Activities of the Root of Taw shauk

Phytochemical Test of Compound

The small amount of pure compound was dissolved in 10 ml of ethanol in test tube. And then this solution was tested with 1 % FeCl3 and 1 % K3Fe(CN)6 solutions. It gave greenish-blue colour. Hence, this compound must be polyphenol compound.

Molecular Formula Determination of Isolated Compound

The molecular formula of pure compound could be examined by ^{1}H NMR, ^{13}C NMR, DEPT and FT-IR spectra.

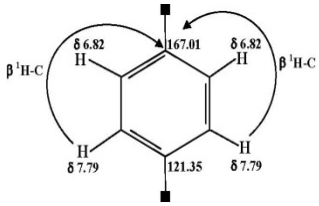
Table (4): Results from ^{1}HNMR,^{13}CNMR, DEPT and FT-IR Spectral of Compound

No.	Information From	No. of C	No. of H	No. of O
1	**^{1}H NMR, ^{13}C NMR, and DEPT spectral data**			
	nine sp2 methine carbons	9	9	
	two methoxy methyl carbons	2	6	2
	one sp3 methylene carbon	1	2	
	eleven sp2 quaternary carbons	11		
2	In FT-IR spectrum, one -OH group		1	1
Total		23	18	3

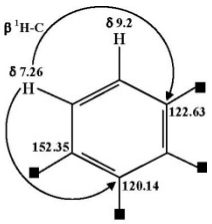
Therefore, the partial molecular formula and the partial molecular mass are $C_{23}H_{18}O_3$ and 242. Information from EI-Mass spectrum, the molecular ion peak is m/z 376. Therefore, the remaining molecular mass is 34. So, the remaining molecular mass 34 should be two -OH groups. Therefore, complete molecular formula was found to be $C_{23}H_{20}O_5$. It is coincident with the "Nitrogen Rule" because of the observation of the even number of molecular mass in EI- MS spectrum.

Structure Elucidation of Isolated Compound

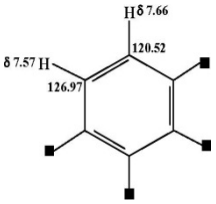
By using the DQF-COSY, ^{1}H NMR and HMBC spectral data, the following fragments **a, b** and **c** could be assigned.



fragment **a**

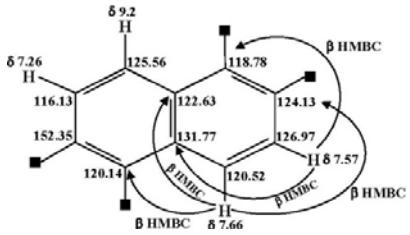


fragment **b**



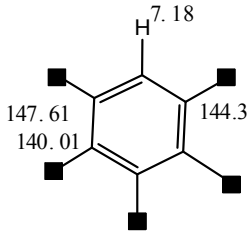
fragment **c**

On the other hand, the existence of β ^{1}H-C long range signals between two aromatic methine protons (δ 7.57 ppm and δ 7.66 ppm) and five sp^{2} quaternary carbons (δ 118.78 ppm, δ 131.77 ppm and δ 120.14 ppm, δ 122.63 ppm, δ 124.13 ppm) in HMBC spectrum leads to the following substituted naphthalene fragment d.



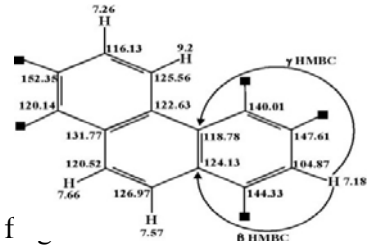
fragment **d**

By using the ^{1}H NMR and HMQC spectral data, the following fragments **e** and **f** could be assigned.



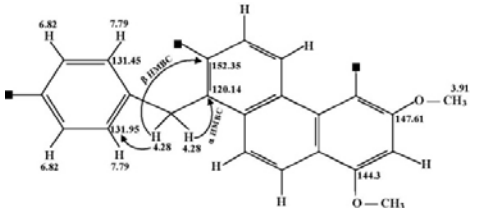
fragment **e**

On the other hand, in HMBC spectrum, substituted anthracene fragment **f** could be observed by the occurrence of β and γ ^{1}H-C long range signal between the aromatic proton (δ 7.18 ppm) and two aromatic quaternary carbons (δ 124.13 ppm and δ 118.78 ppm) in fragment **d.**



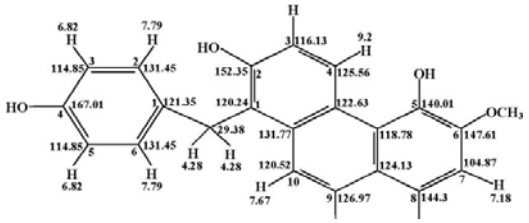
fragment **f**

By using HMBC spectrum, the following extended fragment **g** could be assigned.



Fragment **g**

Due to the logical correlation, the three OH groups should be connected to the three aromatic quaternary carbons with downfield chemical shifts (δ 140.01 ppm, δ 152.35 ppm and δ 167.01 ppm) in fragment **g**. Hence, the complete structure of compound is described as follow.



The IUPAC name of this isolated compound is 1-(4-hydroxybenzyl)-6,8-dimethoxyphenanthrene-2, 5-diol.

4. Conclusion

The root of Taw shauk consists of a variety constituents such as glycoside, phenolic, alkaloid, saponin, polyphenol, flavonoid, steroid and terpene groups. Moreover, the antimicrobial activity tests on ethyl acetate extract of the root of this plant responds high activity on *E. coli* and medium activities on *Staphylococcus aureus, Bacillus pumilus* and *Candida albicans*. Thus ethyl acetate extract of the root of Taw shauk was chosen for isolation of pure compound. The yield percent of this compound is 0.06 % (0.0014 g) based upon the ethyl acetate crude extract. Moreover, in accordance with the phytochemical test on isolated compound, it gives positive phenol test. And then, the antimicrobial activities of the ethanol extract of isolated compound respond medium activities on *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *E .coli*. In addition, the molecular formula of isolated compound is $C_{23}H_{20}O_5$.

Figure FT-IR Spectrum of Isolated Compound

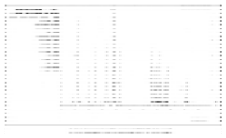


Figure 1 H NMR Spectrum of Isolated Compound

Figure 13 C NMR Spectrum of Isolated Compound

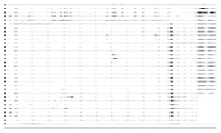


Figure HMQC Spectrum of Isolated Compound

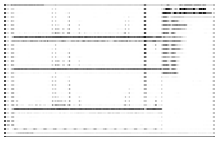


Figure DEPT Spectrum of Isolated Compound

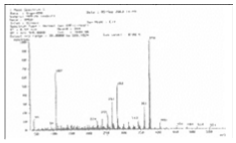


Figure EI-Mass Spectrum of Isolated Compound

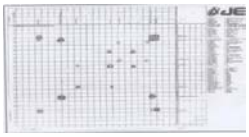


Figure DQF-COSY *Spectrum of Isolated Compound*

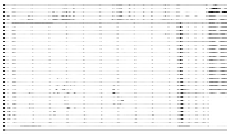


Figure HMBC *Spectrum of Isolated Compound*

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The author would like to thank Professor and Head, Dr Khin Mar Yee and Professor Dr Aye Aye Myint, Department of Chemistry, Mandalay University of Distance Education, Myanmar, for their valuable advice and permission. The author is grateful to Dr Myo Myint, Deputy Director, Fermentation Department, Development Center for Pharmaceutical Technology, Ministry of Industry (1) for his kind support in the test of the antimicrobial activities.

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Tourism Development in a Nature Protected Environment: Indawgyi Lake Area

Zin Mar Than[1], Frauke Kraas[2]

Abstract

The Indawgyi Lake area in the peripheries of Kachin State, Myanmar, is traditionally characterised by three nature-based economic sectors: agriculture, fishery and mining. Because of its specific natural environment, it was designated as "Indawgyi Wildlife Sanctuary" in 1999. Apart from retarding economic development, the area suffers from the conflict between the Myanmar military and the Kachin Independence Organisation. In recent years, Indawgyi Lake is becoming a destination of community involvement in tourism (CIT) aiming at enhancing the local/regional development. These tourism activities need to be in line with the conservation goals. Against this background, the research aims at analysing the current conditions in the area in order to discuss how CIT can become an incentive for a beneficial future development. The empirical analysis is based on a mixed method approach consisting of interviews with experts from relevant sectors (e.g. administration, conservation sector, tourism), field observations and secondary data analysis (primary and secondary statistics). The case study demonstrates that tourism, specifically CIT, can be used as a tool to develop a lagging behind area in concordance with conservation goals. But is still remains a longer way until a satisfying situation of peace will be reached.

Keywords: nature protected area, development, tourism

1. Introduction

The Indawgyi Lake Area in Kachin State, is among those peripheral regions in Myanmar which are economically lagging behind national standards. Up to now, it is dominated by the three nature-based economic sectors of agriculture, fishing and mining. As in many other regions, tourism is seen as a measure to fuel regional development, aiming to improve the living condition of the local people (new work opportunities, increasing income). While its exceptional state of nature – many endemic species exist and are protected in the largest natural inland water reservoir – is a strong development asset, politically the area is instable due to ethnic and resource conflicts. If the future development should be sustainable, it is necessary to protect the natural assets on the one hand while on the other peace negotiations must help overcoming mutual mistrust and fighting between the conflicting parties. Thus, a twofold task is essential for the area: a) the demands of nature protection need to be matched and b) the ongoing, but until yet fragile peace process requires careful attention. Tourism as a development measure thus may lead to two potential benefits: income generation and a successful negotiation of peace between conflicting parties – if a locally adapted, ecologically and culturally sensitive and balanced development will be conceptualised and implemented. A detailed study of the recent tourism-oriented development is yet lacking for the area.

The main aim of the research project thus is to analyse the recent tourism development in the area and to discuss how community involvement in tourism (CIT), a specific form of tourism, can make an important contribution to the future development. Three main questions are in the

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focus of the paper: (1) What are the initial causes for developing tourism in the area? (2) How is the current situation of tourism in the area? (3) What are potentials and possible development paths?

2. Conceptual approach and methods

2.1 Conceptual approach

The three economic sectors – agriculture, fishing and mining – have been facing development inertia over years, as it is elaborated in more detail in 3.1. Introducing industry and manufacture activities seems to be not a realistic solution. For such activities, the region is too far away from connecting urban areas. Insofar, adding tourism as a fourth economic sector seems to be a promising idea to foster development. Two requirements seem inevitable:

1. Because the Indawgyi Lake Area is a nature-protected area tourism has to be organized in a way that it fully meets and optimally supports the requirements of protecting. Eco- , nature- or hiking tourism seems suitable.
2. As the main aim of the new activity is to bring about optimal benefits to the local people without bringing too much external dependency, the idea of community-based tourism (CBT) seems an appropriate development strategy.

A combination of both would be embedded in endogenous regional development approaches. Endogenous development (as described in detail for instance in [1] and [2]) is following the main idea is, that development is initiated and implemented predominantly by local and regional actors and orientated towards the needs and preferences of the local population. At best, it also will lead to a sustainable development, which tries to combine the three main dimensions of sustainability – economy, society, environment – in a way that a future development takes place in concordance with the benefits for the local people and the natural environment of the region.

According to the ASEAN Community-Based Tourism Standard Community-Based Tourism (CBT) is described as: “tourism activity, community owned and operated, and managed or coordinated at the community level that contributes to the well-being of communities through supporting sustainable livelihoods and protecting valued socio-cultural traditions and natural and cultural heritage resources” [3]. In Myanmar the policy of community involvement in tourism (CIT) was issued by the Ministry of Hotels and Tourism in 2013 [4]. Indawgyi Lake Area was listed by the Ministry as one of nine selected destinations. The difference between CBT and CIT (see [5] and [6]) can mainly be seen in the concept that CIT is a less strict form of CBT and the public administration has to approve the activities.

From the perspective of endogenous development, both CIT and CBT fit well conceptually. This holds also true for the expected empowering of the community. Here the chance have to be seen that in conflict areas the different parties can be brought together in order to jointly negotiate and shape a development from which at best all groups involved can benefit. In other parts of Myanmar, namely Thandaung Gyi ([8]), CIT has proven to successfully contribute to the peace building process.

2.2 Methods

This research project employed a mixed method approach (see e.g. [9]), in which qualitative and quantitative information and methods are processed. Altogether 11 expert interviews with members of different relevant institutions and groups – all involved in the Indawgyi tourism development – have been conducted since 2018 (with an average length of between 30 min to more than an hour). The interviews were recorded, transcribed (and translated into English), and entered into the software programme MAXQDA. Thereafter, the data was organized, coded and reflected according to the topic. All interviews received a unique code number (e.g. IDGY-XX, while XX is a consequent number). While the qualitative approach (the expert interviews) was the basic source of information, information on specific aspects was gathered from secondary data and field observation. Secondary data was collected from literature reviews and statistical sources.

3. Research findings and results

3.1 The current situation as starting point for tourism development

In this part the current situation of the area will be looked at from two perspectives: the economy and the natural environment. Additionally, two further aspects, which act as additional circumstances and influence the future development substantially, will be considered, namely the unstable political situation and the conservation status. In more detail, information on the current situation is analysed in [7].

The current economic situation

Eleven village tracts with 33 villages and two towns, Loneton and Nanmun, stretch over an area of 1211.4 km². With about 8,758 households and a population of about 50,000, the Indawgyi Lake area is already quite densely populated.

Agriculture is its major income generating activity. Paddy is the main cultivated crop, followed by peanuts and soy beans. The latter products are mostly grown east of the lake. Most of the agricultural land is located in the lake's fringe areas and around the villages. According to [7] the yield per acre for paddy west of the lake is remarkably lower than in its east (60 baskets to 100 baskets), largely due to the lower quality of the soils.

One of the current challenges of the agricultural sector is a suboptimal land use management, i.e. farmers from the west of the lake practice monoculture cropping systems with one harvest per year, while most of the farmers from east of the lake use multi-culture cropping systems with two harvests per year. As a consequence, the soil quality in the west was degraded gradually and the farmers have to use fertilizer more and more. This is partly also practised by farmers east of the lake, although the natural conditions there are better. That leads in particular to two results: (1) the costs for farming are increasing remarkably, which is a financial burden for the farmers, and (2) eutrophication in the lake is increasing dramatically. Additionally, quite a number of the farms is too small in size, in particular west of the lake [7].

Fishing is the second income-generating activity of the households in the Indawgyi Lake Area and it is mostly a side job for local families. However, fishing is the main economic activity for seasonal migrants from Sagaing and Mandalay Regions and migrants from Shan State. Fishermen need to have a license, issued by the fishery department, for their fishing gears.

Indawgyi fish is famous and consumers prefer them. Therefore, fish and fish products possess a strong market, also outside the region. This might in part be the reason why the number of fishermen has increased in the last years – 560 have been reported in 2017 and 610 in 2018. But only 400 fishermen were registered at the fishery office in 2018, which is a hint for one of the challenges of fishery: overfishing. Other challenges are the wide-spread ignorance of the closed season (from June to August) and the practice of illegal fishing methods (i.e. battery shock, very small mesh size fishing gears). Such infringements are mostly caused by the seasonal in-migrating fishermen, whose number is continuously growing.

Gold mining on a commercial scale has been developed in the area since 1996 ([7]). The activity was very profitable between 1996 and 2002. Today, it is still a popular income generating activity, although mining has decreased in the last years (IDGY-02). According to [7] the threats of the sector can be divided into visible and invisible ones. One visible threat is the sedimentation in the lake caused by the hydraulic method used in gold mining, other visible threats like drug use and health problems are predominant. Invisible, but severe is the threat of mercury contamination, as mercury is used in gold extraction.

In summary, all three economic sectors have shortcomings for income generation and negative impacts on nature and society. Additionally, imbalances in the migration patterns with negative impacts on development exist. The area offers unskilled labour opportunities in the mining and fishery sectors. Simultaneously, educated people have to leave the area and look for qualified jobs in other parts of Myanmar, which is resulting in a substantial brain drain for the region.

The Indawgyi Lake area as a natural environment

Since 1999, a total area of 775 km² including 120 km² of open water area is under conservation as “Indawgyi Wildlife Sanctuary” ([10]). The sanctuary is an ASEAN Heritage site since 2003 and it was designated as Ramsar Site in 2016. In 2017, Indawgyi Wildlife Sanctuary was acknowledged as UNESCO Biosphere Reserve. The main reason for these designations is the rich biodiversity of the lake area, which makes it a unique site: According to [7] altogether 64 fish species are recorded in the lake basin, inflowing streams and marshy areas. Among them, three species are endemic. The lake is one of the most important bird refuges in Southeast Asia, mostly migratory birds; 95 species of water birds have been recorded and the highest number of the Eastern Hoolock gibbons. A detailed list of fauna and flora can be found in [10].

The current conservation status of the area

Beside the lake as a whole, four areas on land belong to the sanctuary. The three big parts are forestland south and east of the lake and a wetland area north of the lake. Additionally, much of the western shoreline is as a small stripe under protection.

Responsible for managing the Indawgyi Wildlife Sanctuary is the Nature and Wildlife Conservation Division of the Forest Department. The designation of the area as a nature reserve makes a zoning system necessary in order to co-ordinate the economic activities and the protecting necessities. A zoning system with three envisaged categories – 1. a core zone, 2. a buffer zone and 3. a transition zone (including agricultural lands, housing areas, small-scale gold

mining areas) – is under process, but until yet not fully implemented. A zoning system is in effect only for the lake (with nine fishing-free zones).

However, some shortcomings have to be mentioned. One is an insufficient number of personnel for fulfilling the duties of conservation, in particular patrolling the area. The patrolling on the land becomes even more difficult because of the unstable political situation, which makes controlling in some areas almost impossible. Another shortcoming is the lack of environmental awareness of the people (i.e. dealing with waste, usage of fertilisers and pesticides). Additionally, a lack of collaboration can be stated, for instance between the fishery department and the conservation side.

The unstable political situation

Since 1962, the Kachin Independence Organization (KIO) has its own army, the so-called Kachin Independent Army (KIA), which has been fighting for autonomy of the state. A branch of this army is located in the area. KIO took part in Aung San Suu Kyi's 21-Century Panglong Conference (= Union Peace Conference), but it did not sign the country's 2015 nationwide ceasefire agreement. For the Indawgyi Lake Area the situation is complicated. It even culminates sometimes into fighting, as for instance in 2018 during the pagoda festival in March. Such an occurrence affects tourism immediately, directly and strongly as an expert (IDGY-03) reported: *"If we have fighting between KIA and Army the bookings are cancelled"*. The prolonged negative effects and uncertainties contribute to the fact that the local people are reluctant to invest much in tourism.

3.2 The current situation of tourism

The uniqueness and attractiveness of the area

The area has a unique natural environment with a rich biodiversity. The specific bird diversity makes the lake spectacular. Moreover, the climate is quite comfortable with moderate temperatures. Together with the beautiful landscape, the quietness and fresh air, the area can become a favourable tourist spot with activities like bird watching, hiking, biking, kayaking and fishing. Additionally, cultural elements like traditional village life, local handicrafts and in particular religious buildings contribute to the attractiveness. The Shwe Myint Zu Pagoda, located in the lake near Nanpade, is an outstanding highlight and the annual pagoda festival (10 days in March) counts up to 80,000 to 100,000 domestic visitors annually.

The current touristic infrastructure

The peak season for tourist activities is from November to March. According to [7], in the period from 2013 to 2015 every year less than 100 international tourists arrived and not more than 150 domestic visitors came to the area (not included the visitors of the pagoda festival). The numbers have increased in the last years. In 2016 about 700 international tourists stayed in the area. In 2017 the number decreased to 580 and in 2018 to 354 – according to an expert (IDGY-03), as a result of the Rakhine conflict. Most of the foreign tourists are from Europe, Japan and Thailand, especially for educational purposes and recreation. Their recreational activities are quite different to those of domestic visitors (IDGY-04). Activities like bird watching, hiking,

biking and kayaking are preferred, while domestic guests are mainly interested in religious attractions.

Until today, foreign tourists are only allowed to stay overnight in Loneton, located on the west side of the lake. Security issues (Loneton has a police station and a military camp) are the main reason for this situation (IDGY-01). Four offers of staying overnight are available: a motel, a guesthouse and two homestays (IDGY-03). The motel has been renovated recently and is nowadays preferred by foreigners because of the better facilities.

The accessibility has become much better in the last years. The road conditions to the outside have been improved as have the ones in the lake area itself. For instance, the main road on the west side of the lake has got a tar surface. Since 2015 also the communication infrastructure has improved quite remarkably. Moreover, the electricity supply has become much better, which for instance is an important element for upgrading of the hygienic situation of the accommodations (i.e. warm water).

An important role in the touristic supply plays the organization Inn Chit Thu, a local civil society organization (CSO). It was established in 2013 supported by the INGO "Flora and Fauna International" (FFI). Nowadays the group has more than 50 members in different villages around the lake, of which about 15 are active. The aim of the organization is described as *"protect and conserve natural environment by participating in social issue"* (IDGY-04). This social issue is very much related to tourism. Inn Chit Thu has an office in Loneton, from where the group offers its tourist services like renting bikes or kayaks. In the office tourists can also get a guide map of the area. At present Inn Chit Thu has three official local guides, who are trained by the Ministry of Hotels and Tourism. Biking and kayaking can be done by the tourists without local guides. For trekking a local guide is needed *"due to landmines and security issue"* (IDGY-04).

At present three settlements take part or plan to take part in CBT/CIT, namely Loneton, Tonsankha and Leponlay: Loneton with a population of 3,400 can be described as the centre for tourism in the lake area. It is located at the main road at the west side of the lake where the transportation accessibility is good. Here the above mentioned CSO Inn Chit Thu has its office and offers services to the tourists.

Tonsankha is quite a small village with only about 100 inhabitants. It is located at the north side of the lake. The accessibility with motorized vehicles is not good. Since 2018, the community is offering lunch for tourists (i.e. bikers, hikers, kajakers) with organic food, which is produced locally. The community intensively wants to develop CBT and is working closely together with Inn Chit Thu (IDGY-04).

Leponlay has a population of about 500. The village is located at the south side of the lake in some distance from the main road. The connection road is in bad condition. Until today the economic base of the village is mainly fishery and some agriculture. Fishery does not work well anymore and CBT is seen as a chance for alternative income (IDGY-05). So, in 2018 the community started to establish CBT. The idea is to offer all needed touristic services (food, vehicles, guide). The food comes entirely from local organic farming. The guide is a member from Inn Chit Thu. In particular, the village tries to build accommodations (for 10 guests) on community land. This project is – in the way it is planned – a good example of CBT. However, it is quite challenging, in particular regarding the accommodation approval and the road

improvement. Regarding the accommodation issue relevant stakeholders (e.g. township administration, parliament member) are supporting the project.

In summary, the initiatives in tourism show positive elements of CBT/CIT. Until now all undertakings are small-scale and locally based. In Leponlay the idea is that a (small) share of profits of private activities (e.g. from handicrafts) is going to the community in order to support other village projects.

Tourism and nature protection – a dual relation

Tourism in the Indawgyi Lake area does have quite a number of characteristics, which are in line with the nature protection aim. Some even support the aims quite remarkably. The following aspects might be named:

- Tourism is staying on a small scale and is a local one. Thus, the number of tourists are limited until now and the locals have an interest to keep the environment attractive.
- At least in Tonsankha and Leponlay cultivation and trade of organic local food is an important element. It supports the idea of nature and living with nature.
- The activities of the tourists are very much orientated to nature (i.e. birdwatching) and carried out in an environmentally sound way (hiking, biking, kayaking).
- The activities of Inn Chit Thu, in particular the guided tours intend to give an idea and understanding of the nature of the lake area to the tourists. In doing so, Inn Chit Thu supports the education aim of the sanctuary in an almost ideal way.
- In the villages, which are dealing with tourism, the people become more aware of environmental and hygienic issues, for instance during trainings. One specific and important aspect is the dealing with waste.

However, it has to be kept in mind that tourism is an economic activity with effects, which might act in contradiction to the aims of nature conservation. In the Indawgyi Lake Area such effects are at least until now not caused by the number of tourists and this probably will also not be the case in future. The people involved in the tourism sector are aware of the threat of too many tourists and try to develop a small scale, CBT-oriented one. An expert stated:

"We do not like to have a big hotel in the village and most of the locals like to develop CBT here" (IDGY-04).

But, problems do exist on land use matters and probably will further intensify in the future. Of course, the most attractive spots are areas with a good view of the landscape (i.e. lake view). This already causes conflicts when accommodation owners want to establish a sitting place in the yard near the lake (IDGY-03). Also the biking path around the lake is a problem because north of the lake the connection is more or less cut off (IDGY-04). This is not convenient for bikers to tour around the lake. The trail is crossing the protected wetland. From the perspective of tourism, it would be desirable to upgrade the trail. From the perspective of nature protection, this is at least questionable.

3.3 Future potentials and development paths – a critical discussion

For the Indawgyi Lake area tourism seems to be the only way, which can have the potential for a further economic sector, which is urgently necessary to improve the life of people. But, tourism should be in a form which meets the needs of the conflicting circumstances located in the region and the needs for nature protection. This requires a limitation of the number of tourists and these tourists should be interested in nature. Thus a kind of combination between ecotourism and CIT seems to be a decisive clue for a prospecting future.

Hiking, biking and boat rides are assumed positive basic activities to explore nature. So hiking paths have to be laid out and at least a biking path around the lake is necessary. At the moment, the main roads might be used for biking. In the future, a separate path, which also can be used by hikers, would be optimal. This is not easy to implement in order to meet the demands of the tourists (close to the lake shore) and simultaneously the necessities of nature protection. Boat rides should be done by ecologically sound boats (e.g. kayaking, rowing or battery/solar driven) with guides, who are aware of the ecology of the lake in order to give information to the tourists. Of course, hiking and biking can be done in groups with a guide. Additionally, self-guided tours should be possible (information signs or boxes with information flyers at points of interest) in order to inform the tourists about nature and culture of the area.

With regard to the idea of accommodation in Leponlay the approval will hopefully be given soon. The project contributes a lot to the idea of community-based tourism and simultaneously supports the aims of protecting nature.

4. Conclusion

CBT/CIT is locally-embedded and corresponds to the idea of endogenous and sustainable development. And it can meet the specific needs of nature protection. A major challenge is to conceptualise an appropriate touristic development for the whole region. Primarily, CBT/CIT is orientated to smaller communities. In the Indawgyi Lake area it has to be organized for a bigger region, in which many villages are located. For sure not all villages can participate intensively because of the given limitations. This may not be a problem at the moment, but will be a challenge in the future, if tourism has been proven as a successful economic activity and more villages want to take part. Thus the entire Indawgyi Lake area needs a detailed plan, which villages should be involved and how CBT/CIT should be implemented. Such a plan has to be jointly developed and discussed openly with the residents in a fair, transparent and trustworthy way. Moreover, this needs to be coupled with sincere negotiations of peace making, which is so far not on the agenda. Probably the conflicting parties, first of all, have to have a deeper mutual interest for going for a ceasefire agreement. This might well be initiated and carried by impulses for a small-scale tourism development, benefiting all parties involved.

Acknowledgements

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Perceived Impacts of Tourism, Quality of Life and Support for Tourism Development at Community Based Tourism in Myaing Township

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Abstract

Community-based tourism (CBT) is a way to promote rural development and hence support poverty alleviation especially for developing countries. This study aims to examine the residents' perceptions on impact of tourism, quality of life and to analyze the effect of their perceptions on support for tourism development at Myaing CBT site in Magway Region. Data was collected from 200 local residents from 1,249 residents at four villages in Myaing using simple random sampling method. According to the results of regression analysis, it was found that residents in Myaing have a perceived high positive and low negative impacts of tourism to have satisfaction on their quality of life, which in turn, all these perceptions of residents affect their support for tourism development. The findings revealed that CBT developers should focus on the well-being of residents and also attract them to actively participate in CBT and create favorable attitudes towards CBT to have their support for future tourism development.

Keywords: Community-based tourism, tourism impacts, quality of life, support for tourism development

1. Introduction

The main purpose of this paper is to address the research gap to comprise local residents' satisfaction of quality of life in considering the impacts of tourism on support for tourism development. This study draws on the effect of positive and negative impacts of residents on CBT at Myaing Township in Magway Region, Myanmar, which is the significant role in contribution to the support of tourism development. In addition, this study investigates whether residents' quality of life influence on additional support to CBT.

CBT is defined as economically, environmentally, socially and culturally responsible visitation to local, indigenous communities to enjoy and appreciate their cultural and natural heritage whose tourism resources, products and services are developed and managed with their active participation and whose benefits from tourism, tangible or intangible, are collectively enjoyed by the communities [1]. CBT is considered as a tool for community development and cultural, natural and environmental conservation. It contributes the approach to help the process of the community development [2] and it has been regarded as a strategy to generate economic gain and to develop the local community, economically and socially.

However, CBT requires the engagement, participation and support of the local residents to make planning, policy implication for additional tourism development, and well operations of current and future CBT development. It is also noted that getting fully support and participation from local residents is the most challenge to the lCBT developers. This challenge seem obvious in Myaing township which is viewed as one of the most conservative CBT sites, where there are full of Myanmar values, traditions, culture, and life style of local community. Myaing CBT is the first CBT project implemented in Myanmar, which has been lasted about 4 years since its

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establishment by Action Aid Myanmar, an INGO operating in Myanmar. Therefore, now is the time to measure its impact on local community and potential for sustainable development in the future. Not only that, in order to get participation and support from the local community, the residents' perception towards the impacts of tourism on their support to the tourism development is a significant issue.

Liu and Var [3] pointed that tourism development is usually evaluated on the basis of economic benefits and challenged on the grounds of social, cultural, and environmental effects. Therefore, the term 'tourism impact' has been paid more and more attention in the tourism literature. It has been the awareness that not only the positive impacts but also negative impacts drive to the support for additional tourism development.

To provide a deeper understanding of the factors affecting residents' support, this study is based on the Social Exchange Theory (SET). Social Exchange Theory has been the most commonly useful framework in explaining residents' responses to tourism development [4][5]. SET considers social interactions as an exchange of resources, suggesting that individuals are likely to engage in an exchange if they expect to gain benefits from it without incurring unacceptable costs [6]. In relation to tourism, residents' perception is built upon their evaluation of tourism "in terms of expected benefits or costs obtained in return for the services they supply" [6]. If the perceived positive impacts (benefits) outweigh the potential negative consequences (costs), residents are likely to support tourism development [7-9]. As such, residents' perceptions of the impacts of tourism are an important consideration for successful operation and development of tourism [10, 11].

In addition, quality of life is one of the key indicators to reflect the prosperity and the well-being of the community [12]. Kim [13] suggested to study the impact of tourism on the overall living conditions of the of tourism destinations, and to examine whether residents who have already received benefits from tourism will support more of the same. Previous researchers found the impact of tourism did have effect on the quality of life for local community [14, 15]. Therefore, the quality of life is the main antecedent of support for additional tourism development [16]. Based on the above literature review, the conceptual framework of the study has been shown in the following Figure. In this framework, community perception on impact of CBT have effect on their quality of life, and then which in turn has effect on support for tourism. There is also direct effects from impacts of tourism on support for tourism development as well.

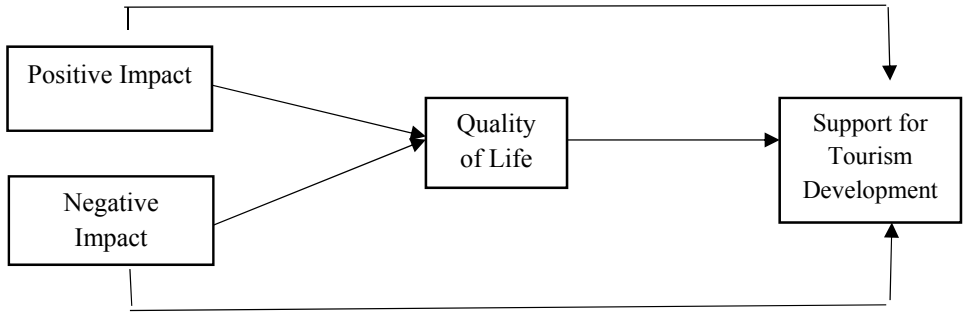

Figure1. Conceptual Framework of the Study

2. Method

This paper used quantitative research method. Both primary and secondary data were applied. Secondary data was collected from the village administrative office of Inn Yaung Village track to know the population of the households; previous research and literature were also collected for literature review. For the primary data, households from four villages (Kan Gyi Taw Village 1 and 2, Su Lae Pan Village, and Inn Yaung Village) participating in CBT in Myaing, Magway Region, in collaboration with Action Aid Myanmar, a non-governmental organization. There were 1,249 households totally in four villages and 200 households were surveyed by personal interview method using structured questionnaire with five-point Likert scale items. Sample households were selected by simple random sampling method from four villages which was assigned proportionately after calculating the required sample size with finite population. The questionnaire was composed of two parts: first part asked the demographic characteristics of the respondent (the household Head or the second most authoritative person in the household) and second part included the questions concerning with the perceived positive and negative impacts of CBT, quality of life and support for tourism. For perceived impact of CBT, it was considered to include from economic, social, cultural, and environment aspects for both positive and negative sides. Positive impacts of CBT were measure by 20 items while negative impacts included 26 items. All of those questionnaires were based on Satarat [17]. Quality of life was measured by formative concept and subjective well-being [18] which included material well-being, community well-being, emotional well-being and health & safety well-being. Material well-being was measured by 7 items. Community well-being was measured by 5 items. Emotional well-being was measured by 5 items. Health & safety well-being was measured by 6 items. For support of tourism from the local residents of CBT, households were asked with 8 items for support of additional tourism [12]. Regression analysis was used to analyze the data in SPSS.

3. Results and Discussion

First and foremost, reliability of the construct variables was checked with Cronbach's alpha value in order to test the internal consistency. It was found that all of the variables were above the 0.7 cut off criteria that they have high reliability. And then, mean values were shown to measure the local residents' perception of positive and negative impacts of CBT, their quality of life and support for additional tourism. Table 1 shows the mean, standard deviation and reliability statistics of all the construct variables. It was found that residents in Myaing had quite high positive perception on CBT as their perceived positive impact was 4.15 and they had very low perceived negative impact of CBT, having 2.22 mean value. Local people in Myaing CBT had moderately high satisfaction on their quality of life, shown by its mean score of 3.87. It was a good point that residents had high level of support for additional tourism in their community (mean = 4.34).

Table1. Mean, standard deviation and reliability of construct variables

	Mean	Standard deviation	Reliability
Positive impact	4.15	0.41	0.815
Negative impact	2.22	0.46	0.842
Quality of life	3.87	0.47	0.865
Support for tourism development	4.34	0.47	0.837

Source: SPSS output (August 2019)

Subsequently, multiple linear regression analysis was run in SPSS to analyze the effect of local residents' perception of positive and negative impacts of CBT on their perceived satisfaction on quality of life. Table 2 results showed that the model was acceptable with R-square value of 0.230. It was found from the result that residents' perceived positive impact of CBT has positive, significant impact on their perceived quality of life satisfaction (β= 0.485, p= 0.000). Based on the instrumental perspective, local residents of Myaing believed that when they have positive benefit from CBT, their quality of life will also be improved. Interestingly, perceived negative impact also had positive but small amount, and significant impact on quality of life satisfaction as well (β= 0.217, p= 0.001). This does not mean that negative benefit of CBT would increase quality of life satisfaction. However, seeing from the low mean value of negative impact, local residents still had very good impression on CBT and they also seemed to be ready to accept the side effect of tourism even though not so willingly.

Table2. The Effect of Positive and Negative Impacts of CBT on Quality of Life

	Unstandardized coefficient		Standardized coefficient	t	Sig
	B	Std error	Beta		
Constant	1.375***	0.327		4.211	0.000
Positive Impact	0.485***	0.072	0.422	6.742	0.000
Negative Impact	0.217***	0.063	0.215	3.434	0.001
F value	29.374***				
R^2	0.230				
Adjusted R^2	0.222				

Source: SPSS output (August 2019)

When testing the regression analysis of residents' quality of life on support for tourism development, quality of life has positive, significant impact on support for tourism development (β= 0.261, p=000). But R-square value was not so high (0.067) that the explanatory power was quite low. The result of simple linear regression was shown in Table 3 as follow.

Table3. The Effect of Quality of Life on Support for Tourism

	Unstandardized coefficient		Standardized coefficient	t	Sig
	B	Std error	Beta		
Constant	3.332***	0.268		12.415	0.000
Quality of life	0.261***	0.069	0.260	3.783	0.000
F value	14.310***				
R	0.260				
Adjusted R^2	0.067				

Source: SPSS output (August 2019)

Even though a lot of factors influence on quality of life, it can be concluded that residents' perception of quality of life has positive influence on support for tourism. Local residents expect higher quality of life to be created by tourism and thus local authorities, CBT initiative committee should ensure that they support them well taking care of the wellbeing of the community. Again, it was necessary to test the direct effect of positive and negative impacts of CBT on residents' support for additional tourism, multiple linear regression was run again in SPSS and the result was shown in Table 4.

Table4.The Effect of Positive and Negative Impact of CBT on Support for Tourism Development

	Unstandardized coefficient		Standardized coefficient	t	Sig
	B	Std error	Beta		
Constant	2.356***	0.288		8.178	0.000
Positive Impact	0.650***	0.063	0.563	10.250	0.000
Negative Impact	-.320***	0.056	-0.316	-5.755	0.000
F value	67.310***				
R^2	0.406				
Adjusted R^2	0.400				

Source: SPSS output (August 2019)

From Table 4, it was seen that when people perceived positive benefits from CBT, they would convey support for additional tourism, being positive, significant effect from positive impact on support for tourism (β= 0.650, p= 0.000). The effect of negative impact of CBT on support was negatively significant that when they perceived negative benefits from CBT, residents would have less support for tourism (β=-0.320, p= 0.000). Since adjusted R^2 was 0.400, it could be concluded that 40% of the residents' support for tourism was explained by their perceived positive and negative impact of CBT on their community. This is in line with the social

exchange theory because according to this theory, people tend to react in the same way they are treated in their perception. Therefore, it is very important for the policy makers to consider creating better positive image of tourism among local community rather than negative ones. While serving the best interest of the tourists, government should not forget the role of the local community and to create better image leaving the positive externalities only to the residents. Finally, to test of mediation effect of quality of life satisfaction on the relationship between positive and negative impacts of tourism on support for tourism, another multiple linear regression was run and shown in Table 5.

Table5. The Mediating Effect of Quality of Life on Impacts of Tourism towards Support for Tourism

	Unstandardized coefficient		Standardized coefficient	t	Sig
	B	Std error	Beta		
Constant	2.194***	0.299		7.341	0.000
Positive Impact	0.593***	0.070	0.514	8.482	0.000
Negative Impact	-.346**	0.057	-0.341	-6.075	0.000
Quality of life	0.118*	0.062	0.117	0.188	0.060
F value	46.646***				
R^2	0.417				
Adjusted R^2	0.408				

Source: SPSS output (August 2019)

Following the Baron & Kenny approach, all the preconditions of the mediation analysis were tested and were significant that, mediation test was proceeded. When quality of life satisfaction was entered into the regression, the effect of positive impact on support was still significant and negative impact either. Thus, it could be concluded that quality of life serves as a mediation on the relationships between impacts of CBT and support for tourism development. Having the positive and negative impacts of tourism could enhance or degrade the quality of life and that can in turn influence the residents' support for additional tourism development.

4. Conclusion

The purpose of this study is to find the effect of positive and negative attitudes towards CBT on the local residents' perceptions of quality of life as well as their support on tourism development. Using the social exchange theory, residents of Myaing CBT were assumed to have their support for tourism development depending on their positive and negative benefits perceived from CBT. This paper also tries to incorporate residents' satisfaction on their quality of life arising from the impact of CBT, to consider the stakeholder perspective and community development as a goal for sustainable tourism development. It was found that local residents of Myaing had a good attitude towards tourism because they have high perception of positive impacts by CBT but a low perception of having negative impacts. The impacts of CBT include

different perspectives such as economic, social, cultural and environmental impacts that this paper tries to include not only economic (egocentric) perspective but also other emotional and social perspectives in considering their response behaviours towards support for tourism. To be more comprehensive, the effect of perceived tourism impacts on residents' satisfaction of quality of life by considering community development as the main target of developing CBT. It was found that residents of Myaing CBT community were also highly satisfied with their quality of life. Under the proper implementation of the CBT project by Action Aid Myanmar and local authorities, people in Myaing CBT area could earn a good material wellbeing, social, and emotional and health and safety wellbeing in their community. They seemed to enjoy the facilities developed directly and indirectly through CBT development, income generation opportunities derived from CBT, the pride to show off their culture while improving the better and safer environmental conditions.

From the logical results, it is found that when people receive positive benefits, they would react with the support for the tourism development but they will not do so when they perceive they have negative externalities. For the sustainable development of community and tourism, it is very important to have local residents' participation and their support. At the same time, tourism should not neglect the benefits of community for sustainable development that this paper is really comprehensive in nature. Through the results, it could be suggested that policy makers, tourism developers should take a balanced approach or triple bottom line approach for implementation of their projects. Tourists are to be served with the best effort of the tour operators, and the industry yet, the satisfaction and wellbeing of the community should never be a failure. Taking care of safe and sound environment is beneficial not only for the tourists but also for the local community concerned. For future researchers, it would be a great to include community attachment and place identity for seeing the big picture.

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Socio-cultural Factors of Pathel Village, Falam Township in Chin State in Myanmar

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Abstract

Chin State is situated in the Western sector of the Republic of the Union of Myanmar, the 13, 907 square miles Chin State is home to Chins. In Myanmar, they predominantly inhabit Chin State, which is located in the Northwest of the country, the bordering Bangladesh to the west and India to the north. The Chin State is divided into two divisions. Northern and Southern During the British Colonial period, the Chin State was under. A Socio-economic study of Falam Township was carried out in 2014. A structured questionnaire was used to collect information. A northern sample of 30 households in 6 villages was selected. A house to house visit was made by 2 interviewers. There are many collections of the historical and socio-cultural evidences of the villages. Out of these villages, the name of the village, Parthel, is explained briefly in this paper.

Keywords: Falam, Cultural, Parthel , interviewers.

1. Introduction

These facts and figures were obtained from the survey of the field trip to Falam, Chin State, Chin Hills, Upper Chintwin. We will glance briefly at the western side of Myanmar, where the hills are occupied by different kinds of Chins. Chin Hills as a whole, the true Chin Hills are the mountainous ones between the Myitha River and the Assam border.

South of this region the Chin Boks, Chin Bone, Yindu and other Chin of the Pakokku Hill Tracts, and the cane, bellied Chins (mogan) of the unadministered Country liner live between Pakokku and Rakhine State, Still further South, at least to Thayet and Pyay are yet other Chins who are more or less Burmanised.

The Chins as a whole are classified as

(i) Northern (Chin Hills Proper)
(ii) Central (Pakokku Hill Tracts) and
(iii) Southern (Minbu, Thayet, Pyay, etc.)

Chin Hills Proper are again divided for administration purposes into Northern, Central and Southern or, if preferred, into Siyin –Thaukkate (Sokte), Tashon and Haka. The Northern Hills (Tiddim) are inhabited by the Thado, Yo, Siyin, Sokte, or Thaukkate and Kamhaung tribes. The central (Falam) by Tashon, Lomban, Laiso, Kwangli, Wheingo and Yahow who are Lai, and, Southern (Haka) by Hsemtong, Zhotung, Lawhtu, Wantu, and by Haka, Yokwa, Ktang Klang, Bwal and Kwatringtlong. The last five are also lai, though the Haka (a group of about ten villages-including the parent village) claim to be the only true Lai.

The Pakokku Hill tracts, of which Kanpetlet is the administrative centre, are divided into a number of great Chin Bok Valleys or Chaungs. These are called the East Maw, West Maw,

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Hnaw, Yaw, Maung, and Kyauksit Chaungs. Further south, round Kanpetlet, are the Salin Paukadu-Piedaw Chaungs inhabited by yintu. Other minor Chin races along the border are the Matu yopa and Mgan. The last are better known to us at "Cane-bellied Chin" from their peculiar dresses which consist only of cane hoops round the stomach. Along the foot of these Chin Hills on the Myanmar side, are curious people called Toungtha and Yawthas or "Children of the yaw" District.

The Yawthas are possibly of Shan Origin, but are now indistinguishable from Burman except for their patois. The Taungthas are probably a hybrid race of Chin origin, though now largely Burmanised. The women still retain a curious national costume. Yindus, but do not now inter-marry with Chins. They are Buddhists, and are believed to have lived at one time on the slopes of Mt.Popa. The second great branch of the migration, and the one we are here concerned with, turned south and overran Myanmar in three main streams Chin, Kachin, Bamar and Lolo. The Chin stream, taking the line of the Chindwin distributed itself along the mountains down the whole length of Myanmar on its western side. A generation ago the Kuki Chins left the Chin Hills, settled in Myanmar and since 1877 have re-entered Myanmar again in the Somara Hill Tracts at a point considerably to the north of their original settlement.

The early history of Myanmar is the story of immigration of the races now occupying it. To observe the Chin tribes, it is known that, three species diverged from Tibet-Myanmar family and they came down into Tibet, Assam and Myanmar. Assam-Myanmar species is comprised of six groups. They are;

(i) Nga-ga group
(ii) Kuki-chin group
(iii) Thet group
(iv) Kachin group
(v) Myanmar group and
(vi) Lolo group

Koki-Chin group spreads in different directions over a wide area such as Somara Hills, Chin Hills and Thandwe. They settle down in forty four localities. Nothern Chins such as Thadaw, Siyin, Kamhaung and Yoe settle down in northern part of Chindwin river. Central Chins such as Le and Lushay or Whelanga settle down in Tiddim, Falam, Haka and Kampetlet.

Southern Chins such as Kyaw, Ahnu, Kaungro live in Rakhine State. Thaung Chin which is included in Southern Chin and to live in Pakokku, Hteelin, Saw and Gangaw township. They weave the famous "Yaw" longyis. She and Uhami; who live in Thayet and Minbu districts are also Southern Chins. Language-affinities have been discovered which, as our knowledge advances, link various people into definite groups. From such evidence only, and by examining the present distribution of the races, it is possible to reconstruct the story of these extraordinary immigrations. These facts and figures were obtained from the survey of the field trip to Falam, Chin State in Myanmar.

The Professor of History Dr Khin Saw Nwe and the Professor and Head of Anthropology Dr Mya Mya Khin jointly conducted a survey of Falam and its neighbouring villages with the permission of Yangon University Asia Research Centre. A Socio-economic study of Falam Township was carried out. A structured questionnaire was used to collect information. A northern sample of 30 households in Six villages selected. A house to house visit was made by

two interviewers. There are many collections of the historical and social-cultural evidences of the villages. Out of these villages, the name of the village, Parthe, is explained briefly in this paper.

2. Chins of Falam

Falam is comparatively well populated. Among Chin Villages Lomban, near Falam, is a pretty large village, and may be taken as typical of the rest. On the opposite slopes across the valley huge settlements of 400 houses are seen covering great areas of mountain side. In a Chin village each tenement is fenced in, leaning a maze of little public paths between the fences. The houses are thatched. The usual trophies hang in the porch. The interior is dark, the roof being hung with corn-cobs, and the floor piled with gourds. These are the chief items of diet. The floor is usually boarded, which gives a superficial appearance of cleanliness.

2.1 Chin languages

The variety of language and dialects in the Chin Hills is bewildering. The language of one village is hardly intelligible to people living a few miles away.

Before the British colonial period incessant and bitter feuds intensified these differences. The several language may, however, be placed very roughly under two groups-Northern and Central-Southern. The languages in each group are basically the same, though mutually unintelligible. However, under British rule, the speech of the most important tribes has assumed a certain currency, so that Kamhow is generally understood in the North, Laizo in the Centre, and Lai in the South. Of these, Laizo and Lai are – sufficiently alike for Falams and Hakas to understand each other with difficulty. The curse of Babel has indeed fallen upon these people. Chins in Government service usually communicate with each other either in Bamar or Hindustani more often the latter. Most of the Siyins and Urdu well.

Nowadays, Chin villagers as well as city people can speak Myanmar language well. Christian missions, pastors and village headmen are implementing social welfare, economy, education and health programmes in Chin villages. The government, local non-governmental organizations, international non-governmental organizations and United Nations Food and Agriculture Organization (FAO) provide assistance on agriculture, water supply, social security, economy, education and health care service in Chin Hills.

The six villages which were visited for the survey are Lonpi, Many Kheny, Zar Linen, Laizo, Lomban Parthe and Cizaymul. We obtained the facts and figures by interviewing and surveying the local people of Chin villages. Why Parthe village is mentioned in the research paper is that it is similar to other villages and ancient, it is also related to traditional culture of Chins. Chin language Parthe is meant by flower.

3. The survey of Falam Township in Chin State

Nowadays, Chin villagers as well as Falam city people can speak Myanmar Language very well. Christian missions, pastors and village headmen are implementing social welfare, economy, education and health programmes in villages. The government, local non-governmental organizations, international non-governmental organizations, and United Nations Food and Agriculture Organization provide assistance on agriculture, water supply, social

welfare, economy, education and health in Chin Hills. Successful attempts can be seen now.

Concerning the economy of the villages, there are rice barns in some villages. Concerning the agriculture, necessary aids have been given to gardening, cultivation, mobile farms and permanent farms. Cabbages, apples, chayotes, oranges, corns, tomatoes and grapes are cultivated in the region and sent to other regions. Cotton weaving, weaving thatch for roofing, making traditional handicrafts of bamboo and rattan can be seen in Chin Hills.

The government as well as other organizations lend loans for the development of agriculture in Chin State. Houses in Chin Hills are chalet built of pine wood. We can see that corrugated iron sheets are used for roofing instead of using thatch. Chalets having long legs can be seen on the mountain slopes. Construction of chalets has improved a lot. In the restaurants, corn and beef which is known as sar-bu-tee and other meals which are cooked with corn are available for three hundred kyats per dish. Aung-lauk pea is also their favourite one.

The stone crosses with names and dates of death on them can be observed on the slopes of the mountains beside the motorway to commemorate the late people. Motorways are being broadened in the whole state so that road communication is gradually improving. In the highway buses, videos which speak Mizo language can be watched. Traditional songs have been converted to modern ones. On the way to Falam, barking deer, wild boars and monkeys inhabit in the jungle. Onion and garlic are grown three times a year in northern Chin State. Yam tuber is widely grown in southern Chin State. Myauk-ngo fruits are exported to India.

Falam used to be the headquarters of the Chin Special Divisional Commissioner. There is the Independence Pagoda at the entrance of the city. There are department of traditional medicine, Public Health Foundation, non-governmental Organization, Religious associations, Myanmar Post and Telecommunication office, courts, office of auditor-general, churches, a general hospital, Basic Education Schools and religious school in Falam.

In observation of the six villages very close to Falam, the author would like to describe Parthel village. It is said that it is a one thousand and fourteen years old ancient village. Parthe village is located in Lonbum village to the east, Lathee and Lomzam villages to the west. The area of it is four square miles and there are four hundred and ninety villagers in ninety households. In summer, there are some courses such as cake baking courses and sewing courses in the village. The young villagers go to Falam for their education. There are not enough midwives in the village. There are also social services such as women's association, and charities.

The villagers help each other in constructing houses. Some relatives of Parthel villagers live in Manipur and India. The transportation is very difficult. In this paper, it will deal with way of life, especially of the Northern Chin people as briefly. But nowadays, as promotions were made in transport and communication of Chin States, travels become easy and smooth in the ways of life in Northern Chin Society. The tribal groups in Myanmar. Thinking and belief were so primitive and it was slowed to develop their society. Like other tribal ethnic people, the chin people have their beautiful culture and way of life. Since ancient times, there have been differences in their history, level of socio-economic development, custom, belief, and religion.

Therefore, the custom, culture, beliefs and language of the different races differ according to their geographical location, climate, and communications. Nowadays we are living a trend of 21st century world. Alone with the changing of the world. Unfortunately, some tribal

languages, cultures, customs and literature have been lost. On the other hand, it is impossible to practice or follow all of the old or ancient traditions in this knowledge age as extremely. However, we have to keep and develop our beautiful cultural resources which may keep us something good for the betterment of our future community. The basic economic needs of the Northern Chins depend on agriculture. A structured questionnaire was used to collect information, 6 villages Selected, Lon pi, Mang Kheng, Zar Linen, Lai zo, Lon yang, Pathe, Cizaymul. There are many collections of the historical and Socio-cultural factors of the villages. Out of these villages, the name of the village, Parthel means beautiful flower.

Parthe village is one of the oldest villages in Falam township. The basic economic needs of the Northern chins depend on agriculture. Parthel village, the basic crops are rice, maize, millet and some vegetables. They raise pigs, chicken, dogs, and mythuns as domestic animals. They use them especially in sacrifice and traditional feasts according to their belief in animism. Rice is the main crop and raw material for "Zu" liquor (rice wine). Marriage is a social obligation in all the chin society. In the village society, the dead bodies are not burnt. The dead bodies were wrapped with clothes and kept in the stone-caves. That place is far from the village on the ridge of the hill. Later, they converted from animists to Buddhism and Christianity. Parthe village's economy is mainly based on agriculture shifting hillside cultivation and terrace farming is used in cultivation. Padauk, Thitya, Ingyin are used for building their houses. Thazin, orchids and honey are also forest product and famous. Hill rice (Sat San) or millet of Northern Chins are also grown.

3.1 Tradition and Customs of northern Chin

Every national races residing in the union of Myanmar has their own traditions and culture. As the chin people are comprised of many tribes, there are also many traditions as well as festivals and dances. But in the present day, it is found that all the festivals cannot be held with the exception of some of them. However, on the significant days of the chins, the festivals are held as the gesture to reveal their culture. It is found that the religion and the traditional cultures of the whole chin region are very similar to those of the Tibeto-Burman group basically that migrated into Myanmar at the earliest times. Only in the later period, there were different kinds of religion in chin state due to the penetration of religions, traditions and cultures from abroad in the successive periods. It is found that the traditions practiced by the chin people in the social ceremonies such as offering and worshipping to the spirits and sacrifices are not so different from other Tibeto-Burman tribes. Sacrifice to their recognized spirits is conducted traditionally and the festivals are held whenever necessary.

The festivals can be divided into three categories such as, economy, social and religion. The festivals concerning with the economy are held with the participation of the entire village. Also in the social festival it can be divided as the festival for happiness and sorrow. Seed sowing festival, harvest festival and traditional festival or eating the first crop and the worshipping festival to the guardian of the farmlands are concerned with the economy. In the religious festival with the exception of the common spirit worshipping festival of the whole village, other worshipping festivals are concerned only with the tribes and are held at any time according to their traditions and customs. The social festivals are the private and are celebrated occasionally.

The Significant festivals are as follows;

1. Kwang Sai pwai
2. Khuo Do pwai
3 Slaying of a tiger
4. Law Ma Annet pwai
5. Kyat Kyar Kok pwai
6. Kyat Ma Phyu pwai
7. Tan Ain pwai
8. Crop high yield festival
9. House warming festival
10. Victory over the Animal
11. Victory over the Enemy

3.2 Khua-Do or New Year festival

Khua Do or New Year festival is held in October after the harvest of the grains grown in the rainy season. The word "Khua Do" is a compound word made up of "Khua" which means village or villagers and "Do" means celebration or entertainment. In other words "Do" refers to the opposition or fighting the enemy. The form of the festival varies from one tribe to another but the ideology and the material objects used in the festival are common throughout all Khua Do in Tidim, Khum Tom in Hkaha. Phan-Ai in Falam varies among spirits believers. Christianity and Phauk chin Hauk believers. However, Khua Do can be divided into two categories. Special Khua Do also known as "Do Mei" lasts four days but it is held every other ten years. The second one is called "Do Mo" or formal Khua Do, lasting three days. During this New Year festival, the chins go to the grave yard in order to pray for the souls or spirits of the deceased ones. In this festival seeking beehive or wasps nest in the forest also plays a profound part. All the youths in the village leave for the forest to seek beehive.

It is believed that if someone dies the wasps would protect the body from insects and worm. The nests of the wasps are broken up and the larvae are taken out and examined very carefully to see what they foretell about the future. If larvae are all healthy and strong, people will be healthy the next year. If the number of larvae in the nets are many, there will be good harvest and plenty the next year. To summarize the reasons for the celebration of "Khua Do" festival are as follows:

(i) To entertain the souls of our departed ancestors whom we invited to the festival

(ii) To drive away all the evil spirits

(iii) To know about the next year through asking the spirit mediums.

Methods

- Group Discussion
- Social Mapping
- Interviewing with native people
- Searching for evidence
- Data collection

Results and Discussion

- This research is to attempt to explain about cultivators from the agricultural villages in Flam township in Chin State.
- How they cope to fulfill their necessities for their livelihood and to analyze and highlight their strong and weak paint.
- Capacity building research for sustainable development and poverty alleviation are prevailing in Myanmar after 2012.
- And then, expected outcome is Chin culture and present life and future prospect and inventive mind, Tourism development can bring native impact of Myanmar society.

4. Conclusion

Every national races residing in the union of Myanmar has their own traditions and cultures. The Chin people are comprised of many tribes, there are also traditions as well as festivals and dances. In the present day, the festivals can be divided into three categories such as, economy, social work and religion. A gentle handshake is a common greeting in Chin Culture. In Chin Language, there are about forty to forty-five dialects of the chin culture. Myanmar embraces all the national races. According to the census of 2014 the population of Myanmar is (51.3) million with the Bamar, the majority races making up about 70%. Falam township in later period becomes as development township in Myanmar. Actually, the Falam township is also near the township of Tamu, bordering with India. Therefore as it was possible to transport and sell the products of Falam township to mainland, the natives'economy was developed.

The basic economic needs of the Northern Chins depend on agriculture. Transportation is very difficult in this region, Nowadays, City Development Committee constructing to the infrastructure. In preserving the traditions and customs in accordance with the policy of the preserving culture, the traditions and customs that are beneficial should be preserved and those which are not appropriate with the time should be forgotten gradually. The youths and the elder persons have already understood this fact. When researchers from foreign countries are interested in Chin traditions and culture, they can come and do research work and it will be of great help for e-tourism in Myanmar.

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Isolation of Piperine from the Fruit of *Piper longum* L. (Peik-chin) and Some Bioactivities of Piperine

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Abstract

The fruit of *Piper longum* L. (Peik-chin) used in the treatment of diarrhea and dysentery in traditional Myanmar medicinal system was chosen for present study. The aim of the study is to isolate active principle from *Piper longum* fruit and to screen some bioactivities: antibacterial and larvicidal activities of isolated compound. The isolated compound, piperine (2.57 g, 1.80 % yield, m.pt. 130 °C) from ethyl acetate extract of *Piper longum* fruit was isolated by solvent extraction, column and thin layer chromatographic methods. It was characterized by some physical properties such as R_f value and melting point besides some chemical properties. The structure of isolated compound was elucidated and identified by modern spectroscopic methods: UV, FT IR, ^{1}H NMR, ^{13}C NMR and FAB mass spectrometry. By agar disc diffusion method, antibacterial activity of piperine was tested on 11 bacteria from clinical source. Minimum inhibitory concentration (MIC) value of piperine was found 0.16 mg/mL on *E. coli* LT assessed by microtitre plate dilution method. The larvicidal activity of piperine was investigated in the range of 0.00625 to 0.1 g/mL by *Aedes* larvae method at Department of Medical Research. Piperine showed lethal concentration activity (LC_{50} = 0.0132 g and LC_{90} = 0.0788 g).

Keywords: ***Piper longum*, piperine, spectroscopic methods, antibacterial and larvicidal activities**

1. Introduction

Diarrhoea and dysentery are important health problems in worldwide especially developing countries. So the Government of Myanmar has initiated a national programme for the development of Traditional Medicine System in combating six major types of diseases: namely; malaria, tuberculosis, diarrhoea, dysentery, diabetes and hypertension. In addition, mosquitoes are the major arthropod vectors and the most dangerous human health pests. In Myanmar, mosquito borne diseases are causing huge loss of life in rural and urban areas and large portion of health fund goes for the treatment and control programs, however mosquito diseases continue to explode from time to time and major public health problem [1]. A larvicide is an insecticide that is specifically targeted against the larval life stage of an insect. Adverse effects of available insecticides on life system desire development of environmental friendly substitute for mosquito eradication program [2]. The application of natural materials as a larvicidal agent for decreasing the harmful effects of chemical pesticides on environment. Chemical control of mosquitoes can cause many unwanted effects on human health and no target animals. By understanding these side effects of chemicals, people are now interested in biopesticides and botanical formulations, which are considered as eco-friendly. Nature is providing numerous bioactive products against vector mosquitoes in the form of plant products and other biological derivatives. In recent years, plant products and phytochemicals have been studied for the control of mosquitoes [3]. In this study, Myanmar medicinal plant, *Piper longum* L. (Peik-chin) shown in Figure 1(a) and (b) was selected to find out of active principle for the treatment of dysentery, diarrhea, and also against larvae. *P. longum* plants are found in the north temperate regions and South East Asia [4]. It is

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cultivated in India, Indonesia, Malaysia, Sri Lanka, Pakistan, Singapore and Bangladesh. *P. longum* is indigenous and grows wild in Myanmar especially in Mon and Kayin States and hilly regions of Northern Myanmar. The pale yellow crystal, piperine is the main constituent responsible for spice and bioactive properties oaf *P. longum* [5]. In Myanmar, *P. longum* is used in treating diarrhea, dysentery, fever, cough, indigestion, stomachaches and asthma [6]. The fruit of *P. longum* has been also used in the treatment of gastrointestinal (GI) problems, pneumonia, tumor, flatulence [7] and then used as spice in food industry like a Nga-yoke-kaung [8]. Therefore, antibacterial activity investigation on EtOAc extract and some isolated phytoconstituents from the fruit of *P. longum* were carried out by using agar disc diffusion method. In this study, Minimum inhibitory concentration (MIC) of active constituent was also determined by microtitre plate dilution method besides the larvicidal activity of active principle was investigated by *Aedes* larvae method.

Figure 1 (a) Plant of P. longum L. *(b) Fruit of P. longum L.*

Botanical Aspects of *Piper longum* L.

Name	:	Peik-chin (in Myanmar), Long pepper (in English)
Botanical Name	:	*Piper longum* L.
Family	:	Piperaceae
Fruit blackish green from	:	Fleshy spikes 2.5 - 3.5 cm long and 5 m thick, oblong, blunt, small shrub [9]

2. Materials and Methods

Plant Materials

The fruit of *P. longum* L. (Peik-chin) was collected from Mawlamyine, Mon State. The plant was identified at Department of Botany, Yangon University. The fruit of *P. longum* was washed, cleaned and dried at room temperature for three weeks. Then the dried sample was powdered and stored in air- tight container.

Instruments: Shimadzu UV-240 (MeOH), Shimadzu FT IR- 8400 (KBr) (at URC), ^{1}H (300 MHz) NMR, ^{13}C (75 MHz) NMR, FAB MS (at University of Goettingen, Germany)

Chemicals : CC; Merck Silica gel 60 (70-230) mesh, eluents; Petroleum ether (PE) - ethyl acetate (EtOAc), TLC; precoated silica gel 60 (F_{254}, Merck)

Extraction and Isolation of Piperine from Fruit of *Piper longum* L.

Preparation of extracts from fruit of *Piper longum* : The air-dried powder (1kg) was individually cold extracted with (2500 mL) of ethyl acetate for 7 days and then filtered. The filtrate was evaporated to dryness at normal pressure on a water bath and desiccated followed by recorded the yield %.

Isolation of phytoconstituents from EtOAc extract of fruit of *Piper longum* : The EtOAc extract was subjected to isolate the active phytoconstituent from Peik-chin fruit by column chromatography. The column was packed with silica gel (400 g) by the wet method using petroleum ether. The column was eluted consecutively with the solvent systems of PE : EtOAc in the ratio of 19:1, 9:1, 4:1, 2:1, 1:1 v/v according to their increasing polarity. Five fractions were monitored by thin layer chromatography (TLC). The fractions that gave similar spots on thin layer chromatography (TLC) plates were combined together and the solvent was evaporated. Finally, pure compound obtained from fraction III, pale yellow crystal (2.57 g, 1.80 %) was characterized as piperine by melting point, TLC determination, UV, FT IR, ^{1}HNMR ^{13}CNMR and FAB MS spectroscopic methods.

Screening of Antibacterial Activity of Ethyl Acetate Extract and Piperine Against 11 Tested Bacterial Strains by Agar Disc Diffusion Method

Agar disc diffusion method was used for the detection of antibacterial activity for ethyl acetate extract from *P. longum* fruit and isolated compound, piperine against 11 tested bacteria from clinical sources, National Health Laboratory (NHL), Yangon; related to acute diarrhea (cholera), dysentery, abscess, pneumonia and typhoid. The test procedure was as follows: the test samples (1 g each) were dissolved in 1 mL of ethyl acetate, and introduced into sterile petridishes for testing 11 cultural bacterial strains. The discs having 6 mm diameter each with 20 μg extract or compound/disc were allowed to dry at 42 $^{\circ}$C in incubator.

The bacterial suspension from trypticase soy broth was streaked evenly into three places on the surface of the trypticase soy agar plates with sterile cotton swab (Puritan, USA). After the inoculums had dried for 5 min, the dried disc impregnated with extract and isolated compound were placed on the agar with flamed forceps and gently pressed down to ensure proper contact. A disc impregnated with solvent only was used as control and antibiotics tetracycline was also used as standard for this study.

After overnight incubation at 37 $^{\circ}$C, the zones of inhibition diameter including 6 mm discs were measured. Then the isolated compound, piperine was tested MIC determination.

Determination of Minimum Inhibitory Concentration (MIC) by Microtitre Plate Dilution Method

The MIC value of isolated compound, piperine from *P. longum* was tested with five strains; *E. coli* EHEC, *E. coli* LT, *E. coli* EPEC, *S. aureus* ATCC and *S. aureus* WS by microtitre plate dilution method. It was done by using trypticase soy broth by dissolving with appropriate soluble compound, piperine in 2-fold dilutions. First, an inoculum of pure culture of respective bacteria was seeded in 5 mL of trypticase soy broth (TSB) and incubated at 37 $^{\circ}$C for

3-4 h to obtain a turbidity of 0.05 by MacFarland nephelometer which corresponded to a bacterial suspension of 10^6 organisms per mL. Prior to the experiment, 50 L of TSB was introduced into all wells of 96-well microtitre plate. The compound (piperine) was dissolved in ethyl acetate and diluted with trypticase soy broth to obtain the following concentration: 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, 0.32 mg/mL, 0.16 mg/mL, 0.08 mg/mL, 0.04 mg/mL, 0.02 mg/mL, 0.01 mg/mL, 0.005 mg/mL, 0.0025 mg/mL in 96 - well microtitre plates. Then 0.02 □L of the prepared inoculum was introduced to its respective wells and the microtitre plates were incubated at 37 °C for 18 h. The contents of all wells were thoroughly mixed with a multi-channel pipetter to re-suspend clamped cells at the bottom of the wells in a solution. Growth of the bacteria was determined by automated microplate reader (Bio Rad) at a wavelength of 450 nm as well as confirmed by the growth of culturing onto trypticase soy agar to incubate at 37 °C for overnight. The concentration of the compound in the last well with no growth of bacteria was taken to represent the minimum inhibitory concentration (MIC) of the compound.

In vivo larvicidal activity of Piperine on Aedes aegypti mosquito larvae

Hlaingthaya Township strain of *Aedes aegypti* mosquito larvae and adult *Aedes* mosquitoes emerged from pupae were reared in laboratory of Medical Entomology Research Division, Department of Medical Research. Larvae and adult mosquitoes emerged from larva survey were identified by morphological methods [10]. Larvae were fed on DMR larva food. Adult were provided with 10 % sucrose solution and 8 weeks old mouse for blood meal. Mosquitoes were held at 26 ± 2 ºC, 65-75 % relative humidity with a photo period of 12-h light and 12-h dark. Laboratory reared larvae were used for testing insecticidal properties of piperine by WHO standard method. Different concentration of piperine from *P. longum* fruit was prepared as 0.1 g, 0.05 g, 0.025 g, 0.0125 g and 0.00625 g in 100 mL each of distilled water in 150 mL plastic cups respectively. Fifty (50) each 3rd and 4th instars *Aedes aegypti* larvae were put into different concentrations and also negative control test was done simultaneously. Detail testing was performed by WHO standard method [11]. Larvae were exposed 24 h for each replication in different concentrations samples in laboratory at 27-29 ºC and 70 to 80 % relative humidity. Five replicates were carried out and knockdown was checked after 60 minutes. Then, mortality was recorded after 24 h of exposure period. Knockdown and dead larvae were identified when the larvae failed to move after probing with a needle in the thorax region of the body. Lethal concentration LC_{50} and LC_{90} values for 95 % confidential limits were calculated by chi square equation. Data entry and processing was made using Microsoft Excel software. The average larval mortality data were subjected to probit analysis for calculating LC_{50} and LC_{90} values and other statistics at 95 % confidence limits of upper confidence limit and lower confidence limit, and chi-square values were calculated using the dose-effect probit analysis [12]. Results with Probability $p < 0.05$ were considered to be statistically significant.

3. Results and Discussion

Isolation and Characterization of Piperine from Ethyl Acetate Extract of *P. longum* Fruit

The dried fruit powder collected from Mawlamyine, Mon State was extracted with ethyl acetate solvent and the yield % of ethyl acetate extract (4.5 %) was obtained. Piperine (1.80 %) was isolated from ethyl acetate extract by column chromatographic separation method using petroleum ether : ethyl acetate solvent system. The isolated compound as piperine was then

identified by melting point, TLC determination, UV (Figure 2), FT IR (Figure 3), ^{1}HNMR (Figure 4-a and b), ^{13}CNMR (Figure 5-a and b) and FAB MS (Figure 6) spectrometry. Melting point of the isolated compound was found to be 130 °C which was identical with reported value of piperine [13]. Isolated compound gave R_f value 0.20 with petroleum ether - ethyl acetate (4:1) system and was observed yellow colour with anisaldehyde-conc. H_2SO_4 reagent after heating in TLC chromatogram.

Piperine : Pale yellow crystal (2.57g,1.80%yield); m.pt. 130°C; λ_{max} 309,340 (nm); FT IR (cm^{-1}) 3009(ν -$_{CH}$ aromatic), 2940, 2920, 2862 (ν- $_{CH\ asym\ \&\ sym\ of\ -}$ CH_2) , 1634 (ν $_{C=O}$), 1612 (ν C=C asym & sym diene), 1584, 1491(ν C=C aromatic), 1449(δ - CH_2), 1253, 1194 ($\nu_{asym\ C-O-C}$), 1032, 1018 ($\nu_{sym\ C-O-C}$), 1134 (δ-$_{CH}$ of phenyl), 997 (δ-$_{CH}$ of trans CH=CH group), 847, 831, 805(δ_{CH} out of plane bending); ^{1}H NMR (300 MHz, $CDCl_3$) / δ_H (ppm), 1.55 ~1.68 (6H, m, H2, H3, H4), 3.55, (4H, broadS, H1, H5), 5.95(23H, S, H10), 6.43(^{1}H, d, J=12Hz, H6), 6.73(^{1}H, d, J=12Hz, H12), 6.749 (^{1}H, S, aromatic H), 6.77 (^{1}H, d, J = 6Hz, aromatic H), 6.87- 6.90 (^{1}H, dd, H7), 6.955 (^{1}H, d, J=6Hz,aromatic H), 7.37-7.44(^{1}H, dddd, H13); ^{13}C NMR and DEPT (ppm); 24.7 (3C, C14, C13, C17), 26.08(2C, C15, C16) 101.19(1C, C12), 105.58 (1C, =C=CH aromatic), 119.91(1C, CH=C= aromatic), 122.42 (1C, C2), 125.26 (1C, C4), 130.92(1C, C6), 138.47 (1C, C5), 142.97(1C, C3), 148.04 (1C, C8 aromatic), 148.10 (1C, C12), 165.35(1C, C1)(amide); FAB MS⇨ m/z 286.3[M+H^{+}], 308.1[M+Na^{+}], 594[2M+Na^{+}], 877 [3M+Na^{+}] 285.3 [∴M$^{+\cdot}$], 285[$C_{17}H_{19}NO_3$] (Figures 2,3,4 and 5) [14]

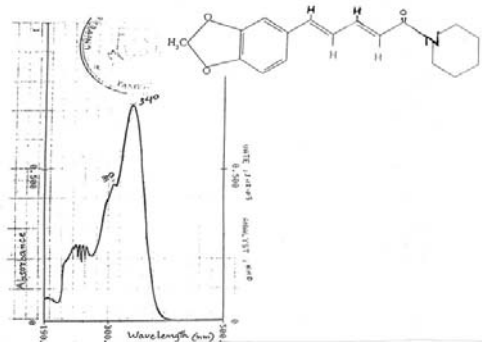


Figure2. Ultraviolet spectrum of isolated compound from fruit of P. longum (MeOH)

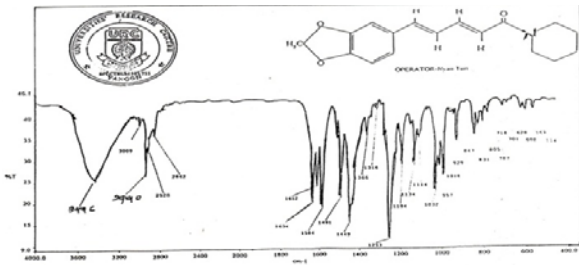


Figure3. FT IR spectrum of isolated compound from fruit of P. longum (KBr)

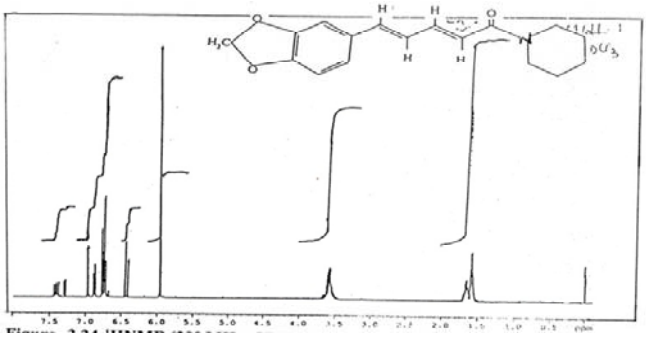


Figure 4(a). ^{1}HNMR (300 MHz, $CDCl_3$) spectrum of the isolated compound from fruit of P. Longum

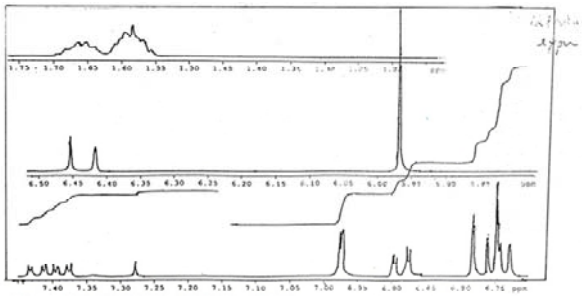


Figure 4(b). ^{1}HNMR (300 MHz, $CDCl_3$) spectrum of the isolated compound from fruit of P. Longum

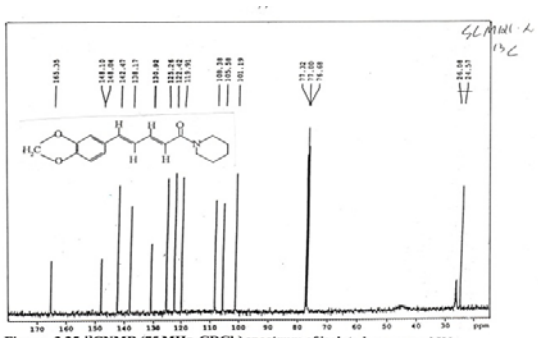


Figure 5(a). ^{13}CNMR (75 MHz, $CDCl_3$) spectrum of isolated compound from P. longum

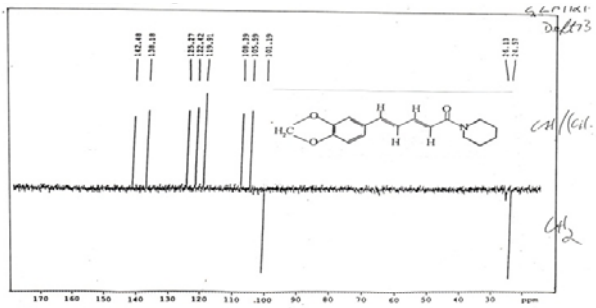


Figure 5(b). ^{13}CNMR (DEPT) spectrum of isolated compound from P. longum

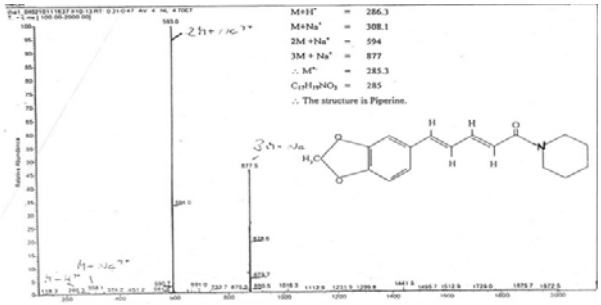


Figure6. FAB mass spectrum of the isolated compound from fruit of P.longum

Screening of Antibacterial Activity of Crude Extracts and Isolated Piperine

Screening of antibacterial activity of ethyl acetate extract and isolated piperine were being compared on 11 tested bacteria from clinical sources shown in Table 1 and Figure 7. In Table 1 it was found that ethyl acetate extract exhibited more potent to 11 strains with inhibition zone diameter (13-24) mm whereas the isolated piperine showed less potent activity with inhibition zone diameters (8-25) mm of all strains except *Pseudomonas aeruginosa* and *Proteus species*. According to these zone diameters, the antibacterial activity of both ethyl acetate and piperine against *Klebsiella* species, *Salmonella typhi* and *Proteus species* are more potent than tetracycline (standard). From the screening results, it can be generally deduced as follows. The EtOAc extract and isolated piperine from *P. longum* were found to inhibit the tested bacteria with regard to acute diarrhoea (cholera), dysentery, pneumonia, typhoid, urinary tract infection, gastrointestinal (GI) infection, sepsis and abscess. In addition, piperine yielded (1.80 %) from *P. longum* was employed by microtitre plate dilution method for minimum inhibitory concentration (MIC) determined with 2 strains of *Escherichia coli* and 2 strains of *Staphylococcus aureus* obtained from clinical sources at Bacteriology Research Division, DMR (LM) (Table 1 and Figure 8). The microtitre plate dilution method also elaborates the specificity, sensitivity and the least amount required for media, reagents and glassware. It also saves time and working space in conducting the experiments. The minimum inhibitory concentration (MIC), 0.16 mg/mL of piperine was the most potent to *Escherichia coli* LT showed that the plant possess potent bactericidal activity on it. From the MIC elucidation, piperine isolated from the fruit of Peik-chin would be more effective for the treatment in diarrhoea, (GI) infection and abscess. It has antibacterial action against *E. coli* responsible for diarrhea as well as gastrointestinal (GI) infection and *Staphylococcus aureus* responsible for abscess occurred in skin, mouth and nose.

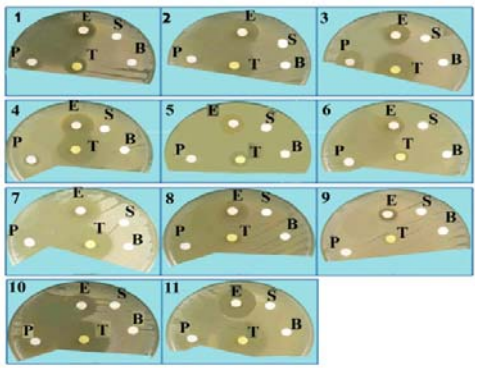
Table1. Antibacterial Activity of EtOAc Crude Extract and Piperine, Isolated from Fruit of P. longum

Sample	Inhibition zone diameter (mm)										
	1	2	3	4	5	6	7	8	9	10	11
EtOAc Crude Extract	14	20	19	21	21	18	23	18	13	20	24
Piperine	15	8	19	11	-	24	24	25	-	19	20
Blank	-	-	-	-	-	-	-	-	-	-	-
EtOAc solvent (control)	-	-	-	-	-	-	-	-	-	-	-
Tetracycline (standard)	13	25	25	25	15	10	25	12	-	29	29

Tested Bacteria (From Clinical Sources*)

1 =	*Klebsiella* species	8	=	*Shigella flexneri*
2 =	*Salmonella paratyphi* A	9	=	*Proteus* species
3 =	*Citrobacter* species	10	=	*Staphylococcus aureus* WS
4 =	*Escherichia coli* EHEC	11	=	*Vibrio cholerae* O1
5 =	*Pseudomonas aeruginosa*			
6 =	*Salmonella typhi*			Disc diameter = 6 mm
7 =	*Escherichia coli* LT			- = no activity

***** National Health Laboratory (NHL), Yangon



1 =	*Klebsiella* species	8	=	*Shigella flexneri*
2 =	*Salmonella paratyphi* A	9	=	*Proteus* species
3 =	*Citrobacter* species	10	=	*Staphylococcus aureus* WS
4 =	*Escherichia coli* EHEC	11	=	*Vibrio cholerae* O1
5 =	*Pseudomonas aeruginosa*			
6 =	*Salmonella typhi*			
7 =	*Escherichia coli* LT			

E = EtOAc crude extract (Peik-chin)

P= Piperine B = Blank

S = EtOAc solvent (control) T = Tetracycline (standard)

Figure7. Antibacterial activity of EtOAc crude extract and piperine isolated from P. longum (Peik-chin)

Table 2 Minimum Inhibitory Concentration (MIC) of Active Isolated Compound Piperine (mg/mL) of P. longum

No.	Bacteria	MIC(mg/mL)
1	*Escherichia coli* EHEC	0.32
2	*Escherichia coli* LT	0.16
3	*Escherichia coli* EPEC	0.32
4	*Staphylococcus aureus* ATCC	0.625
5	*Staphylococcus aureus* WS	0.625

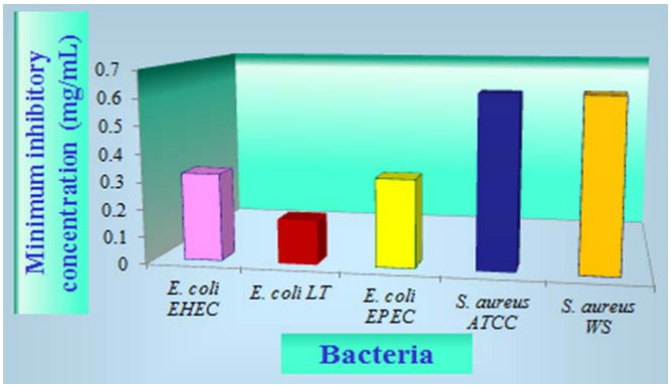


Figure8. Minimum inhibitory concentration (MIC) of active isolated compound piperine

Examination of Larvicidal Activity of Piperine Isolated from *P. longum* Fruit against 3rd and 4th Instars *Aedes Aegypti* Larvae

Larvicidal activity of piperine isolated from *P. longum* fruit was examined by WHO standard method at Department of Medical Research (DMR), Yangon. From the results of current study, it was denoted that the lowest knockdown effect was occurred at 0.00625 g/100 mL dilution of piperine with the 12.8 % of knockdown. In addition, the highest knockdown effect of *Aedes aegypti* larvae was performed in 68.8 % knockdown at 0.1 g/100 mL dilution. Furthermore, the percent in the lowest mortality effect for piperine was 25.6 % at 0.00625 g/100 mL dilution. And then, the highest mortality effect of *Aedes aegypti* larvae was found out 92.4 % mortality at 0.1 g/100 mL dilution of piperine. The doses of 50 % mortality (LC_{50}) value of piperine against 3rd and 4th instars *Aedes aegypti* larvae was found in 0.0132 g /100 mL and also 90 % mortality (LC_{90}) value of piperine was found to be 0.0788 g/100 mL.

4. Conclusion

From the fruit of *P. longum* L., 4.5 % of EtOAc extract was obtained and then isolated 1.80 % of pale yellow crystal by using column chromatographic separation followed by identified as piperine with the use of melting point, TLC determination, UV, FT IR, ^{1}HNMR, ^{13}CNMR and FAB MS spectrometry.

In vitro antibacterial activity of ethyl acetate crude extract and piperine was also investigated. It was found that inhibition zone diameter of EtOAc extract ranged between (13-24) mm whereas piperine showed the range of inhibition zone diameter between (8-25) mm. It may be concluded that the antibacterial activity of the crude EtOAc extract was more potent than that of piperine, pure compound against 11 tested bacteria; *Klebsiella* species, *Salmonella paratyphi* A, *Citrobacter* species, *Escherichia coli* EHEC, *Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli* LT, *Shigella flexneri, Proteus* species, *Staphylococcus aureus* WS and *Vibrio cholerae* O1. They are effective for the treatment of dysentery, urinary tract infection, food poisoning, abscess and diarrhoea.

Minimum inhibitory concentration (MIC) values of piperine against five tested bacteria were determined by using microtitre plate dilution method. Nevertheless, MIC values of piperine showed against all five tested bacteria: *E. coli* EHEC, *E. coli* LT, *E. coli* EPEC, *S. aureus* ATCC, *S. aureus* WS, it was the most potent to be 0.16 mg/mL against *E.coli* LT. In addition, the larvicidal activity of piperine fruit was investigated in the range of 0.00625 to 0.1 g/100 mL by *Aedes* larvae method at Department of Medicinal Research (DMR). From these observations, the highest knockdown of *Aedes* larvae was found at concentration of 0.1 g/100 mL of piperine. The highest mortality rate (92.4 %) of *Aedes* larvae at the concentration of 0.1 g/100 mL of piperine. Among them, piperine showed the highest lethal concentration activity (LC_{50} = 0.0132 g/100 mL and LC_{90} = 0.0788 g/100 mL). From these observations, it may be recommended that the ethyl acetate extract of fruit of *P. longum* (Peik-chin) and isolated piperine may be used as main materials for the traditional medicine formulation in the treatment against dysentery, gastrointesinal tract infection, food poisoning, abscess and diarrhea. From the larvicidal activity test, it could be suggested that the isolated compound, piperine may have the potential to be further developed into alternative source of mosquito control agent.

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