STUDY ON SOME HEAVY METAL CONTENTS, NUTRITIONAL VALUES AND ANTIMICROBIAL ACTIVITY OF RHIZOME OF *COSTUS SPECIOSUS* (KOEN.) SM.

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Abstract

Costus speciosus (Koen.) Sm. is locally known as Phalan-taung-hmwe and belongs to the family Costaceae. In this research, the plant was collected from Hpa-an Township, Kayin State during June to October, 2017. In this study, atomic absorption spectrophotometer (AAS) analysis, nutritional values and antimicrobial activity of rhizome of *Costus speciosus* (Koen.) Sm. were undertaken. The content of heavy metals was analyzed by using Atomic Absorption Spectrophotometer(AAS). In according to the results of atomic absorption spectrophotometer, only cadmium (0.004 ppm) is found among heavy metals. Therefore, the level of cadmium in *Costus speciosus* (Koen.) Sm. was below the standard limit considered safe for human consumption and did not give harmful effect on human health. The experiment for the nutritional values of *Costus speciosus* (Koen) Sm. was carried out at the Food Industries Development Supporting Laboratory (FIDSL), Yangon. According to the results, the carbohydrate content in rhizome was higher percentage than others. In antimicrobial activities, the different solvent extracts were investigated with six types of microorganisms by using agar well diffusion method. According to this experiment, ethyl acetate extract showed most significant antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis* and *Candida albicans.*

Keywords: *Costus speciosus* (Koen.) Sm., Heavy metal contents, nutritional values and antimicrobial activity.

Introduction

The plant *Costus speciosus* (Koen.) Sm. belongs to the family Costaceae. The native of plant is Indo-Malayan region, occurring from India to New Guinea (Rodriguez, 2005). *Costus speciosus* (Koen.) Sm., locally known as Phalan-taung-hmwe in Myanmar and Indian spiral ginger in English (Hundley and Chit Ko Ko, 1987; Kress *et al.*, 2003).

A large number of plants have been used as traditional medicine in many parts of the world. Among them, *Costus speciosus* (Koen.) Sm. plays an important role as an herbal medicine for the treatment of various health ailments. In general, plant based medicines are safe and there are no side effects compared to other drugs and more effective treatment of health disorders (Malabadi *et al*., 2016).

Costus speciosus (Koen.) Sm. is used in traditional medicine in the treatment of fever, expectorant and cough (Ashin Nagathein, 1968). Leaves and young stems are used internally for diarrhea, eye and ear infections. Decoction of stem is used to control fever and dysentery (Duraipandiyan, *et al*., 2012). In Myanmar, the juice of stem is used for ear infection and otorrhoea (San Hla, 1960). The roots are peeled and placed on the infected tooth for an hour to provide relief from toothache (WHO, 2009). The roots are used to treat cough, dyspepsia, skin disease, worms, snake bite, aphrodisiac, purgative, anthelmintic, febrifuge, expectorant, and catarrhal fever (Malabadi *et al.,* 2016).

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The rhizome of *Costus speciosus* (Koen.) Sm. may be beneficial in protection and alleviation of diabetic complications. The rhizomes are bitter, astringent, cooling, aphrodisiac, purgative, anthelmintic, expectorant, tonic and useful in burning sensation, constipation, worm infection, skin diseases, fever, asthma, bronchitis, inflammations, anemia, improve digestion, pneumonia, urinary diseases and jaundice (Verma and Khosa, 2012).

The human body requires a number of minerals for their growth and other activities which are obtained from plants, since plants absorb and accumulate minerals from the environment which is necessary for its growth. Plants can also accumulate metals from the environment. Environment, pollution, atmosphere, soil, harvesting and handling are some of the factors, which play a major role in contamination of medicinal plants by metals and also by microbial growth. Therefore, it is necessary to measure and establish the levels of metallic elements in the herbal plants as these elements when consumed at higher levels become toxic (Gajalakshmi, *et al*., 2012)

Nutrition is a science that studies all the reactions that occur between living organisms and food. Food includes plant and animal products that when consumed, can yield energy and provide nutrients needed to maintain life and allow growth and reproduction (Grosvenor and Smolin, 2002). The human body requires substantial amounts of some nutrients, particularly those that will provide energy and support growth and development of the body tissues, namely carbohydrates, fat, and protein, as well as water (Williams, 1999).

Microorganisms are living things so small that they can be seen only with the aid of microscope. They are widely distributed in nature and are responsible for many physical and chemical changes importance to the life of plants, of animals and of human beings (Sarles, *et al*., 1956).

The aim and objectives of this research are to analyze the content of some heavy metals in powdered drug, to investigate the nutritional values, and to examine the antimicrobial activities of the different solvent extracts from rhizomes of *Costus speciosus* (Koen.) Sm.

Materials and Methods

Collection and preparation of powdered samples of *Costus speciosus* (Koen.) Sm.

The specimens of *Costus speciosus* (Koen.) Sm. was collected from Hpa-an Township, Kayin State during June to October, 2017. The collected samples of rhizomes were thoroughly washed with water to remove impurities. After washing the samples, they were cut into small pieces then air dried at room temperature for several days. When constant weight was obtained, the dried samples were pulverized by grinding machine to get powder and stored in airtight containers for further studies.

Determination of some heavy metals by Atomic Absorption Spectrophotometer

The ash samples were used to study the content of heavy metals and analyzed by using Atomic Absorption Spectrophotometer according to the method of Levinson (1974) at the Applied Geology Department, University of Yangon.

Preparation of sample for Atomic Absorption Spectrophotometer (AAS)

In this study, Perkin Elmer Analyst 800 spectrophotometer was used. Ten grams of powdered sample was placed in a weighed crucible and heated in a Muffle furnace at 300ºC to

achieve complete ash. About 0.5 g of ash was filtered with 80 meshes and digested in 5 ml of HNO_3: HCl (1: 4) concentrated acid mixture. The solution was evaporated overnight to dryness in air. The residue was leached on a water-bath treated with 10 ml of HNO_3 weak acid mixture at a temperature of about 70ºC for 30 minutes and then 10 ml of deionized water were added. The solution was stirred by using vortex mixer. The resultant solution (10 ml) was pipetted accurately and made up to 100 ml with deionized water again. The solution was stand for overnight and then aspirated on an atomic absorption spectrophotometer.

Nutritional values from the rhizome of *Costus speciosus* (Koen.) Sm.

The analysis for the nutritional values of rhizomes of *Costus speciosus* (Koen.) Sm. was carried out at the Food Industries Development Supporting Laboratory (FIDSL), Yangon. The nutritional value had been investigated according to the method AOAC (Horwitz, 1980).

Antimicrobial activities of different solvent extracts from rhizomes of *Costus speciosus* (Koen.) Sm.

Antimicrobial activities of different solvent extract from rhizomes of *Costus speciosus* (Koen.) Sm.were tested on six pathogenic microorganisms by using agar well diffusion method at the Central Research and Development Center (CRDC), Yangon.

Preparation of crude extracts from rhizomes of *Costus speciosus* (Koen.) Sm.

The dried powdered rhizomes (5g) of *Costus speciosus* (Koen.) Sm. was extracted with 50ml of petroleum ether, chloroform, ethyl-acetate, acetone, ethanol, methanol and distilled water respectively for seven days and then filtrated by using filter paper. The filtrate solvents were evaporated on a water bath. All these extracts were used for the determination of antimicrobial activities.

Test organisms

The test organisms used were *Bacillus subtilis, Bacillus pumalis, Candida albican, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus. These* microorganisms are listed in Table 1.

Table 1 Test organisms, their respective code numbers and diseases

Test organisms	Code number	Diseases
Bacillus subtilis	N.C.I.B-8982	Food spoilage and fever
Bacillus pumalis	N.C.T.C-8236	Eye infection, soft tissue infection
Candida albican	IFO-1060	Pathogenic, vaginal candidiasis, alimentary tract infection, cardiac infection, skin infection, intestinal tract infection and vagina mucosa, sinus irritation, intense itching, sores and inflammation
Staphylococcus aureus	N.C.P.C-6371	Food poisoning, boils, abscesses, headache, skin infection, wound sepsis, burns, muscle cramping, nausea, vomiting, illness,
Escherichia coli	N.C.I.B-8134	Diarrhoea and vomiting, dysentery, urinary-tract infections,
Pseudomonas aeruginosa	N.C.T.C-6749	Urinary-tract infections, surgical wound respiratory infection, soft tissue infection, bone and joint infections, central nervous system infection, gastrointestinal infection, chronic lung, burn infection, ear infection, eye infection, bacteremia and septicemia.

Preparation of sample for testing antimicrobial activity

Screening of Antimicrobial activity of crude extracts has been done by agar-well diffusion method. Nutrient agar was prepared according to the method described by Cruickshank (1975). Nutrient agar was boiled and 20-25 ml of the medium was poured into each conical flask, plugged with cotton wool and autoclaved at 121 °C for 15 minutes. Then the conical flask was cooled down to 40-45 °C and poured into sterilized petridishes and each of 0.1-0.2 ml of test organisms were also added into the dishes. The agar was allowed to set for 2-3 hours. After this, 10 mm agar-well was punched with the help of sterilized agar-well borer. Then, about 0.2 ml of sample was introduced into each agar- well and incubated at 37 °C for 24 hours. The inhibition zone appeared around the agar-well, indicates the presence of antimicrobial activity. Then the diameter of inhibitory zone was measured with the help of a transparent ruler. At the same time, the controlled experiments using solvent only were prepared for the comparison with rhizomes extracts.

Results

Determination of some heavy metals by Atomic Absorption Spectrophotometer

The content of heavy metals (As, Hg, Pb and Cd) were analyzed in the powdered rhizome samples by using AAS, measured in the unit of ppm. According to the results of AAS, the contents of elements cadmium (Cd) was found as 0.004 ppm but arsenic (As), mercury (Hg) and lead (Pb) were not found in the rhizome. The results were shown in Table 2.

Table 2 Some heavy metals in *Costus speciosus* (Koen.) Sm. rhizome.

No.	Elements	ppm
1.	Arsenic (As)	ND
2.	Mercury (Hg)	ND
3.	Lead (Pb)	ND
4.	Cadmium (Cd)	0.004

Nutritional values from the Rhizome of *Costus speciosus* (Koen.) Sm.

The nutritional values of *Costus speciosus* (Koen.) Sm had been undertaken according to the method AOAC (Horwitz, 1980). In this experiment, the amount of protein content 3.47%, crude fiber content 20.06%, crude fat content 1.60% and carbohydrate content 51.71% were observed in *Costus speciosus* (Koen.) Sm. rhizomes. Therefore, the carbohydrate content in rhizome was higher percentage than others. The results were shown in Table 3 and Figure 1.

Table 3 Nutritional values from the rhizome of *Costus speciosus* (Koen.) Sm.

No.	Types of Nutrients	Content (%)
1.	Protein	3.47
2.	Crude Fiber	20.06
3.	Crude Fat	1.60
4.	Carbohydrate	51.71

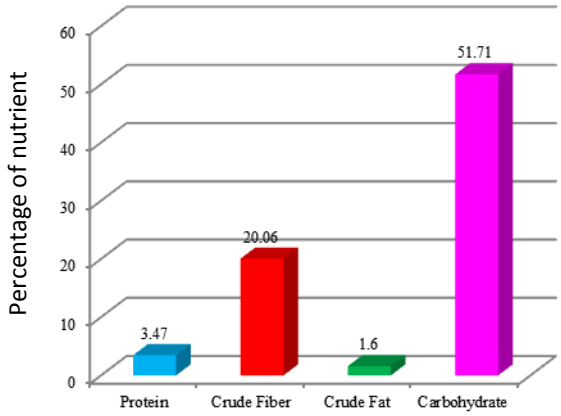

Figure 1 Nutritional values from the rhizome of *Costus speciosus* (Koen.) Sm.

Antimicrobial activities of different solvent extracts from the rhizomes of *Costus speciosus* (Koen.) Sm. by using agar well diffusion method

The antimicrobial activities were tested on six types of pathogenic microorganisms by using agar-well diffusion method. According to this experiment, ethyl acetate extract of rhizomes showed the most significant antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis* and *Candida albican but* non against *Escherichia coli.* Chloroform extract of rhizomes indicated moderate antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus,* weak *against Escherichia coli* and *Pseudomonas aeruginosa.* Methanol and ethanol extracts showed moderate antimicrobial activity against *Pseudomonas aeruginosa*, weak activity against *Bacillus subtilis* and *Staphylococcus aureus.* Acetone extract showed weak antimicrobial activity against *Bacillus subtilis, Pseudomonas aeruginosa* and *Bacillus pumalis.* Petroleum ether extracts exhibited weak antimicrobial activity against *Bacillus subtilis* and aqueous extracts showed moderate against *Staphylococcus aureus* but none against other microbes. The results were shown in Table 4 and Figures 2 to 8.

Table 4 Antimicrobial activities of different solvent extracts from the rhizomes of *Costus speciosus* (Koen.) Sm.

Solvents	***Bacillus subtilis***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Bacillus pumalis***	***Candida albican***	***Escherichia coli***
Petroleum Ether	14 mm	-	-	-	-	-
Chloroform	15 mm	15 mm	11 mm	-	-	13 mm
Ethyl acetate	45 mm	40 mm	38 mm	44 mm	20 mm	-
Acetone	12 mm	-	12 mm	11 mm	-	-
Methanol	13 mm	11 mm	19 mm	-	-	-
Ethanol	12 mm	11 mm	19 mm	-	-	-
Distilled water	-	17 mm	-		-	-

Agar well~ 10 mm

10 mm~14 mm (weak activity), 15 mm~19 mm (moderate activity), 20 mm~above (high activity)

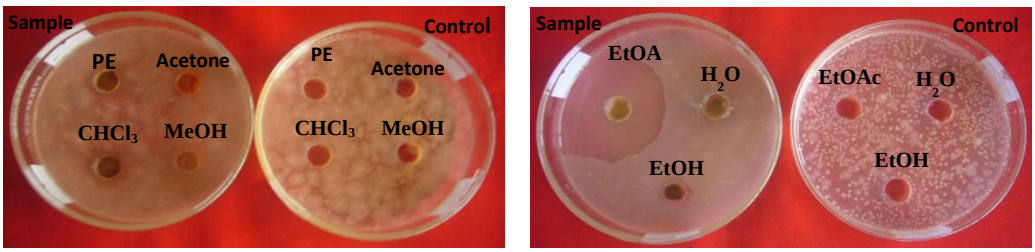

Figure 2 Antimicrobial activities of rhizomes of *Costus speciosus* (Koen.) Sm. againsts *Bacillus subtilis*

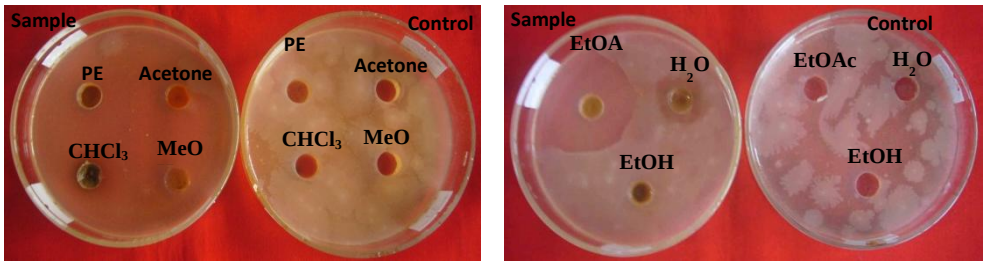

Figure 3 Antimicrobial activities of rhizomes of *Costus speciosus* (Koen.) Sm. againsts *Staphylococcus aureu*

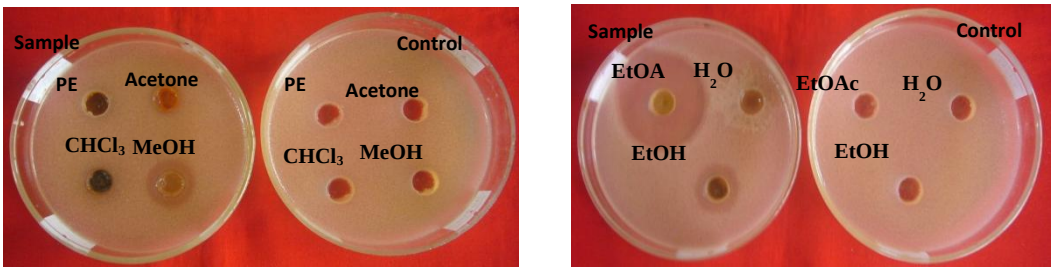

Figure 4 Antimicrobial activities of rhizomes of *Costus speciosus* (Koen.) Sm. againsts *Pseudomonas aeruginosa*

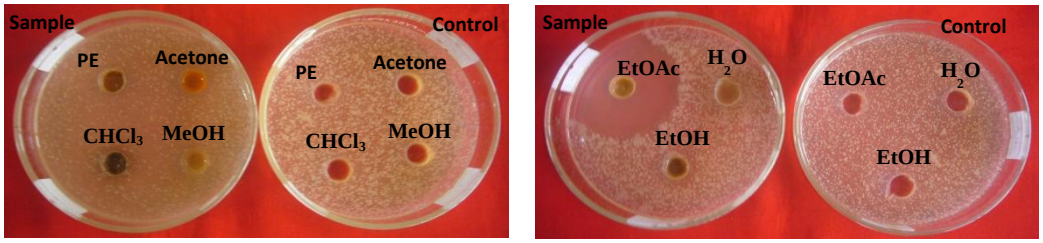

Figure 5 Antimicrobial activities of rhizomes of *Costus speciosus* (Koen.) Sm. againsts *Bacillus pumalis*

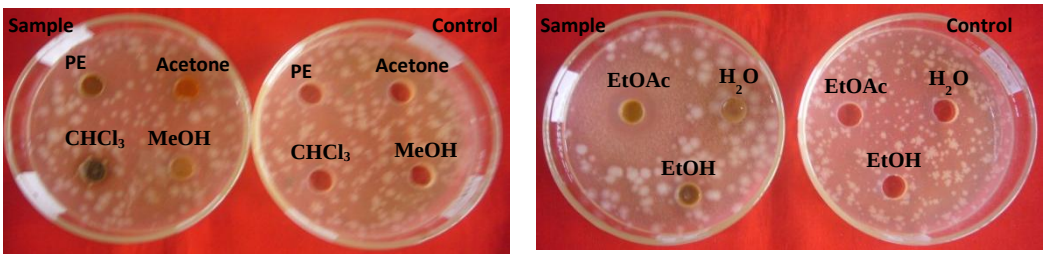

Figure 6 Antimicrobial activities of rhizomes of *Costus speciosus* (Koen.) Sm. againsts *Candida albican*

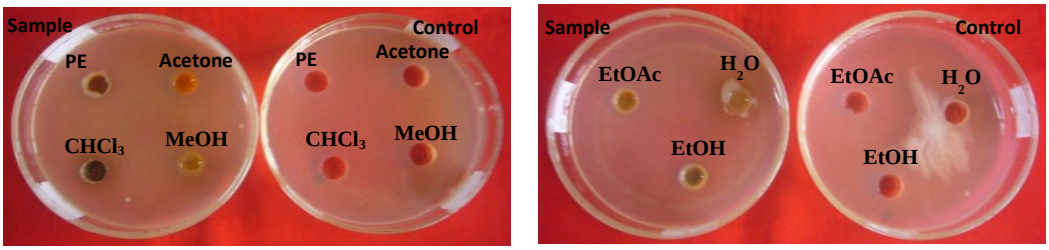

Figure 7 Antimicrobial activities of rhizomes of *Costus speciosus* (Koen.) Sm. againsts *Escherichia coli*

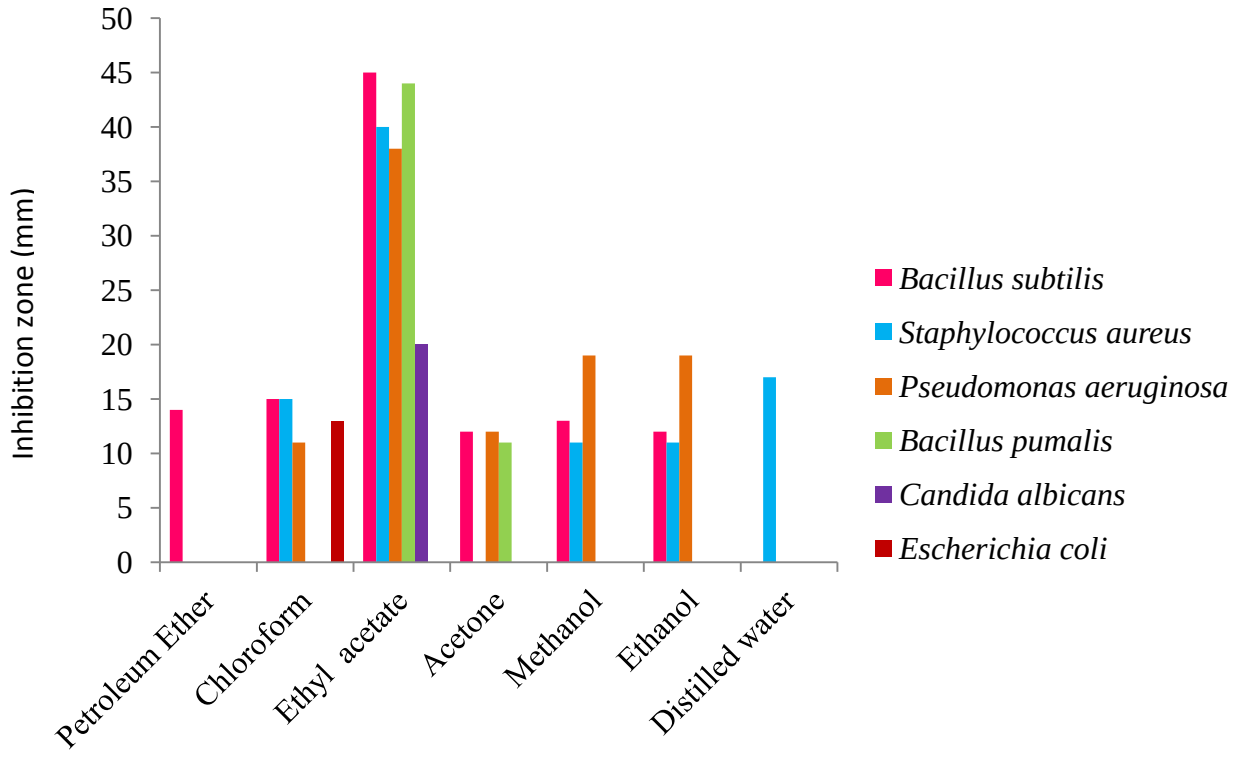

Figure 8 Antimicrobial activities from the rhizome of *Costus speciosus* (Koen.) Sm.

Discussion and Conclusion

In this research, some heavy metal contents, nutritional values and antimicrobial activity of rhizome of *Costus speciosus* (Koen.) Sm. were investigated.

According to the results of (AAS), the contents of elements cadmium (Cd) was found to be 0.004 ppm but arsenic (As), mercury (Hg) and lead (Pb) were not found in the rhizome of *Costus speciosus* (Koen.) Sm.

The ingestion of cadmium exceeding 15 mg kg^{-1} body weight (bw) may give rise to gastrointestinal symptoms such as vomiting, abdominal cramps and diarrhoea, whereas doses of 20-30 mg kg^{-1} bw have caused human fatalities. The lowest emetic dose reported is 0.07 mg kg^{-1} bw (Bull, 2010). The Food and Drug Administration limits the amount of cadmium in food colors to 15 parts of cadmium per million parts of food color (15 ppm) (http://extoxnet.orst.edu/faqs/foodcon/ cadmium.htm). According to Bull (2010) and WHO (http://extoxnet.orst.edu/faqs/foodcon/ cadmium.htm), the level of cadmium in *Costus speciosus* (Koen.) Sm. was below the standard limit (<0.07mg/kg body weight and 15 ppm respectively) considered safe for human consumption and would not have harmful effect on human health. WHO (2007) recommended permissible limit for cadmium in medicinal plant is 0.3 mg/kg. In this study, cadmium in rhizome of *Costus speciosus* (Koen.) Sm. was below WHO permissible limit for cadmium. So, the rhizome of the plant cannot give harmful effect on human health.

According to the result of nutritional values, the amount of protein content 3.47%, crude fiber content 20.06%, crude fat content 1.60% and carbohydrate content 51.71% were found to be present in *Costus speciosus* (Koen.) Sm. rhizomes. The carbohydrate was present as major

constituent than others. Therefore, *Costus speciosus* (Koen.) Sm. rhizomes possess a greater amount of carbohydrate content.

Carbohydrates are the main source of energy for the body. Human body can store only limited amounts of carbohydrates. Excess carbohydrates are converted and stored as fat. The major function of carbohydrates is to provide energy for bodily functions. This energy is needed to carry on body processes such as breathing, maintaining body temperature, and contraction and relaxation of the heart and muscles (Meeks *et al.,* 2009).

In the results of antimicrobial activities, all solvent extracts such as petroleum ether, chloroform, acetone, ethyl-acetate, ethanol, methanol and aqueous extracts of *Costus speciosus* (Koen.) Sm. rhizomes showed different antimicrobial activities on six test microorganisms (*Bacillus substilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis, Candida albicans* and *Escherichia coli*).

Duraipandiyan *et al*., (2012) stated that chloroform, ethyl acetate and methanol extracts from the rhizomes of *Costus speciosus* (Koen.) Sm. showed antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis* and *Candida albicans* but according to the results of this research, ethyl acetate extract of rhizomes showed the most significant antimicrobial activity against *Pseudomonas aeruginosa, Bacillus pumalis* in addition to *Bacillus subtilis, Staphylococcus aureus*, and *Candida albicans*. Chloroform extract of rhizomes indicated moderate antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus*, weak effect against *Escherichia coli* and *Pseudomonas aeruginosa*. Methanol and ethanol extracts showed moderate antimicrobial activity against *Pseudomonas aeruginosa*, weak effect against *Bacillus subtilis* and *Staphylococcus aureus*. Acetone extract showed weak antimicrobial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Bacillus pumalis*. Peteroleum ether extract exhibited weak antimicrobial activity against *Bacillus subtilis* and aqueous extract showed moderate effect against *Staphylococcus aureus*. Therefore, ethyl acetate extract showed more significant antimicrobial activity than other extracts on six test organisms.

In conclusion, the rhizome of *Costus speciosus* (Koen.) Sm. could be used for the treatment of urinary tract infection, respiratory system infection, gastrointestinal infection, asthma, fever, ear infection, eye infection, skin infection, worm infection, inflammation, sores and burn infection and illness which are caused by microorganisms.

Acknowledgements

I wish to express my deep gratitude to Dr Mi San Mar Lar, Professor and Head and Dr Thandar Soe, Professor, Department of Botany, Dawei University for giving their permission to present this paper.

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