STUDY ON NUTRITIONAL VALUES, PHYSICOCHEMICAL PROPERTIES, ANTIMICROBIAL ACTIVITY AND CYTOTOXICITY OF PREPARED NUTRACEUTICAL TABLET FROM SELECTED FRUITS

Aye Aye Mar[1] , Aye Chan Nyein[2]

Abstract

In the present work locally grown *T. Chebula* (Phan -kha), *P. emblica* (Zibyu) and *T. bellerica* (Thit-seint) were selected to make nutraceutical tablets. The selected fruit samples were identified at Botany Department University of Yangon. Then the nutritional values, mineral contents, Physicochemical properties, effect of storage time, phytochemical constituents, antimicrobial activity and cytotoxicity of the prepared tablet samples were determined. The water content of 29.94 %, ash content of 4.00 %, protein content of 1.89 %, fiber content of 2.22 %, fat content of 3.04 %, carbohydrate content of 58.91 %, in the tablet sample energy value of 270.56 kcal/100g and vitamin C content of 44.88 mg/100g were observed. The pH of the prepared sample was 4.20 and the total acidity of tablet contained 2.08 mg/100 g . The effect of storage time on water contents, ash contents, vitamin C contents, total acidity contents and pH of prepared tablet samples were also determined. It was found that, the water content slightly decreased from 1 month to 3 month storage time duration. The ash also slightly decreased and the vitamin C content significantly decreased within 3 months duration time. The total acidity slightly decreased and pH values were not changed with the longer storage time. The mineral contents of prepared tablet samples were determined by ED XRF spectrum. It was found that 0.549 % K, 0.138 % Si, 0.118 % Ca, 0.024 % P, 0.021 % S, 0.008 % Fe, 0.003 % Mn, 0.002 % Rb, 0.002 %Cu, 0.002 % Sr and 0.001 % Zn were present in this sample The preliminary phytochemical tests indicated that various types of secondary metabolites such as alkaloids, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, terpenoids and organic acids together with α -amino acids and starch were present in the sample. The antimicrobial activity of tablet samples from medicinal plant species has been evaluated *in vitro* against microorganisms including five bacterial species (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus and Escherichia coli*) and one fungus species (*Candida albicans*). In general ethanol and watery extracts of tablet samples exhibited antimicrobial activity. The cytotoxicity of water extracts of nutraceutical tablets was evaluated by brine shrimp cytotoxicity bioassay. The crude watery extract was not cytotoxic to brine shrimp up to maximum dose of 1000 µg/ L. The LD_{50} value of standard $K_2Cr_2O_7$ was <1 µg/L and caffeine was > 1000 µg/L.

Keywords: nutraceutical tablet, the acidity, antimicrobial activity, cytotoxicity

Introduction

Nutraceutical Tablets

The term nutraceutical was coined from nutrition and pharmaceutical in 1989 by Stephen Defelice, founder and Chairman of foundation for innovation in medicine, an American organization which encourages medicinal health. Restated and clarified in press release in 1994, its definition was “any substance that may be considered a food or part of a food and provides medical or health benefits, including the prevention and treatment of disease. About 2000 years ago, Hippocrates emphasized ‘Let food be your medicine and medicine be your food’s. The actual use of Nutraceuticals is to attain desirable therapeutic outcomes with reduced side effects.

[1] Dr, Assistant Lecturer, Department of Chemistry, University of Yangon
[2] MSc, Department of Chemistry, University of Yangon

Such products may range from isolated nutrients, dietary, supplements and diets to genetically engineered 'designer' foods, herbal products and processed foods such as cereals, soups, and beverages (Swaroopa and Srinath, 2017).

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

Figure 1 Fruits of (a) *Terminalia bellerica* (Thit-seint) (b)*Terminalia chebula* (Phan-kha) (c) *Phyllanthus emblica* (Zibyu)

Health benefit and traditional uses of Thit-seint

Fruits are laxative, astringent, althelmintic, and antipyretic; Useful in hepatitis, bronchiyis, asthma, dyspepsia, piles, diarrhoea, coughs, hoarseness of voice, eye diseases and scorpion-sting and hair tonic. Half ripe fruit is used as purgative and kernel of the fruit is narcotic. Plants and plants parts are used in the traditional system of medicines like Ayurveda, Siddha, Unani and Chinese medicine (Anindita *et al*., 2016).

Health benefit and traditional uses of Phan-kha

It exhibit a number of medicinal activities due to the presence of a large number of different types of phytoconstituent. It is used as a traditional medicine for household remedy. It is also extensively used in Ayurveda, Unani and Homoeopathic medicine and become a cynosure of modern medicine. Sometimes it is referred to as 'Mother of all healing' (Anwesa *et al*., 2013).

Health benefit and traditional uses of Zibyu

The fruits are sour, astringent,bitter, acrid, sweet and cooling.It is useful in vitiated conditions of tridosha, diabetes, cough, asthma, bronchitis, cephalalgia, ophthalmopathy, dyspepsia, colic.It is also used to cure skin diseases, leprosy, haematoenesis, inflammations, anemia, emaciation, hepatopathy, jaundice, strangury, diarrhoea, dysentery, emorrhages, leucorrhoea, menorrhagia, cardiac disorders, intermittent fevers and greyness of hair (Rohit *et al*., 2012).

Materials and Methods

Samples

Thit-Seint (*Terminalia bellerica*), Phan-Kha (*Terminalia chebula*) and Zibyu (*Phyllanthus emblica L*.) fruits were collected from Magway region and Kyun Kalay Village, Hlegu Township, Yangon Region.

Materials

Analytical grade reagents were used.

Methods

Nutraceutical tablets were prepared as follows. Mature fresh fruits were washed with water and cut into small slices with the knife. The cut fruits were dried in sunlight and ground with blender. The pure and fine fruits powder was obtained. Next, 10 g of each fruits powder was placed in a beaker and dissolved in 300 mL distilled water. Then 50 g of honey was added in this mixture. This mixture was stirred thoroughy on hot plate until fine paste and tablets were made by hand. Then the nutritional values of prepared tablets were studied. The moisture content of tablet samples was determined by oven drying method. Ash content by Muffle Furnance Method, protein content by Dumas Nitrogen analyzer (NDA 701 analyzer , S -42 Department of Chemistry, UY), fiber content by Acid- based digestion method, carbohydrate and energy content by AOAC (2000) methods, mineral content by EDXRF technique and vitamin C content and total acidity by titration method. The reducing sugar was determined by Fehling's solution test and the preliminary phytochemical investigation was carried out by test tube method. Antimicrobial activity of prepared tablet samples were studied by agar well diffusion method and the cytotoxicity of water extract of prepared tablets was evaluated by brine shrimp cytotoxicity bioassay.

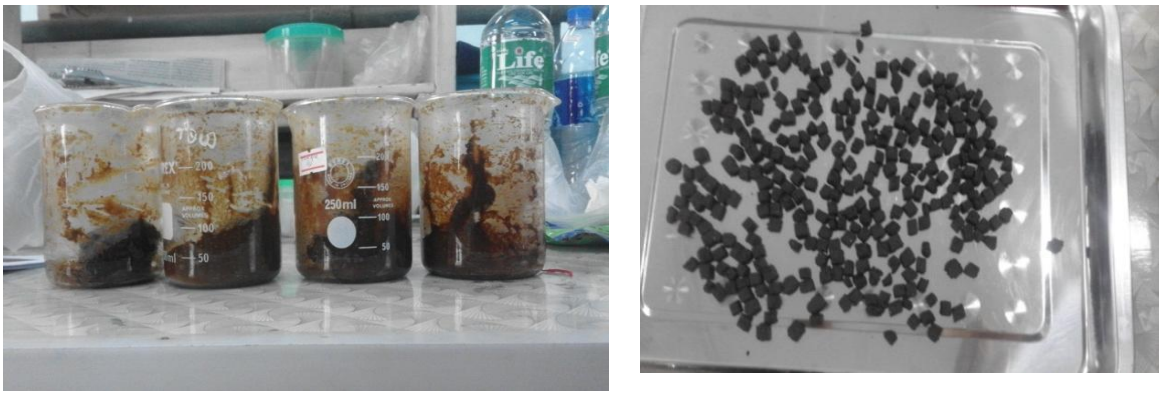
Results and Discussion

Nutraceutical Tablet Samples

In this research, nutraceutical tablet samples were made from three different fruits. The pure and fine nutraceutical tablet samples were prepared by mixing powder of three fruits and honey (Figure 2 and 3).

(a) Zibyu (b) Thit-seint (c) Phan-kha

Figure 2 Pure and Fine fruits Powder



(a) Fine paste sample (b) Tablets

Figure 3 Fine paste sample and Tablets

Nutritional Values of Nutraceutical Tablets

In the present work , water content of nutraceutical tablets were determined by the use of an electric oven at 105 ˚C by drying to obtain constant weight and taking the loss in weight as water. In this study, water content was determined to obtain percentage of water in tablets.

The water content of prepared sample was 29.94 % (Table 1). Water content is one of the most commonly measured properties of food materials. The propensity of microorganisms to grow in foods depends on their water content. For this reason, many foods are dried below some critical moisture content. The texture, taste, appearance and stability of foods depend on the amount of water they contain.

The quantity of ash was determined in order to calculate the carbohydrate content by deduction method. In determining ash, the sample was heated to a high temperature, so that all organic matter is oxidized and volatilized and the mineral are remained. The ash content of prepared nutraceutical tablets was 4.00 % (Table 1).

Various methods have been employed for quantitatively estimation the amount of proteins in biological samples. In this research, NDA 701 Dumas Nitrogen Analyzer was used to determine the protein content of prepared tablet samples and found to be 1.89 % (Table1).

Results from various studies have demonstrated that adequate fiber intake has many additional health benefits and may prevent or decrease an individual's risk of developing coronary disease, stroke, hypertension, diabetes, obesity and colon cancer. In this study, the fiber content of prepared tablet samples was 2.22 %. The result is shown in Table 1.Increase fiber intake may also lower serum cholesterol levels and blood pressure (Anderson *et al*., 2009).

Total fat content was determined by extracting the dried material with petroleum ether (40-60℃) in a continuous extraction apparatus of the Soxhlet type. The fat content of prepared nutraceutical tablet samples was 3.04 % (Table 1). Carbohydrate is one of the main dietary compounds. The carbohydrate content of the diet was calculated by difference.

Carbohydrate % = 100- [protein (%) + water (%) + ash (%) + Fiber(%)]

The carbohydrate contents of the prepared tablet samples was 58.91 %. (Table 1).

Food rich in carbohydrate provide high amount of energy. Carbohydrate can be oxidized to furnish energy and glucose in the blood is a ready source of energy for the human body. The energy content of nutraceutical tablet was 270.56 kcal/ 100 g (Table 1).

Table 1 Nutritional Values of the Prepared Nutraceutical Tablets

No.	Test Parameter	Content
1	water content (%)	29.94
2	Ash (%)	4.00
3	Protein (%)	1.89
4	Crude Fiber (%)	2.22
5	Crude fat (%)	3.04
6	Carbohydrate (%)	58.91
7	Energy value (kcal/100 g)	270.56

Physicochemical Properties of the Prepared Nutraceutical Tablet Samples (pH, Total acidity, Vitamin C content, Reducing sugar content)

pH is one of the important parameters for human diet. Food quality and the stabilization of food colour is mainly due to the pH effect. In this research, the pH content of the prepared tablet samples was 4.2. The result is shown in Table 2. The fluctuations of pH might be due to the variations in titratable acidity.

The organic acids present in foods influence the flavour, colour, prevent the growth of microorganisms or inhibit the germination of spores and providing the proper environment for metal in chelation, an important phenomenon in the minimization of lipid oxidation (Nielsen, 2014). In this study total acidity of 2.08 mg/100 g was observed in prepared nutraceutical tablets. The results are shown in Table 2.

Vitamin C is one of the substances that contribute to the antioxidant capacity in food.. About 30 % of the Vitamin C present in fresh fruit is destroyed during the sample making process, but that which remains in the finish product is stable during storage (Ozkan, 2004). Vitamin C content of nutraceutical tablet samples was determined by iodometric titration and 44.88 mg/100 g vitamin C content was obtained. The result is shown in Table 2.

A reducing sugar is any sugar that is capable of acting as a reducing agent because it has a free aldehyde group or a free ketone group. All monosaccharides are reducing sugars, along with some disaccharides. Reducing sugars react with amino acids in the maillard reaction a series of reactions that occurs while cooking food at high temperature and that is important in determining the flavor of food (William, 1993). The reducing sugar content is determined by Fehling's solution test. It was found that the reducing sugar content of nutraceutical tablet samples was 1.65 % (Table 2).

Table 2 Physicochemical Properties of the Prepared Nutraceutical Tablets

No.	Test Parameter	Content
1	pH	4.20
2	Total acidity (mg/100 g)	2.08
3	Vitamin C (mg/100 g)	44.88
4	Reducing sugar (%)	1.65

Time of Storage

Time of storage is mainly effect on water, ash, pH, acidity and vitamin C content. The high water content in the foods make suspectible to mold damage.

Storage temperature has an important role because this reduces or inhibits the speed of all physicochemical, nutritional and microbiological processes, and thus prolongs, storage period. The storage temperature should be below 2℃ , lower temperature (0.10℃) help maintain taste, colour and water dehydration ratio and also to some extent vitamin C.

The effect of storage time on water content of prepared tablet samples

Water contents of the prepared tablet samples are shown in Table 3 and Figure 3. Storage time effect the water content of prepared tablet samples. There was no changed in the physical quantities of the samples stored at room temperature. The water content ranges from 29.94 % to 28.49 %, 26.59 % and 20.89 % after 1 month, 2 months and 3 months respectively, of storage time.

The lower the water content showed that they would have better keeping quality.

The effect of storage time on ash content of prepared tablet samples

An important determination of the analysis of a food is the estimation of the amount of ash it contains. The ash or inorganic nutrients are the total of non- combustible substances of food.

In this study, the results of the ash content showed that there was gradual decrease in the quality parameters as the storage period increase. The values of the ash contents on the fresh prepared tablet was 4.0 %. After 1 month, 2 months and 3 months of storage time, the values were 3.9 %, 3.1 % and 3.0 %, respectively.

The effect of storage time on pH prepared tablet samples

The effects of storage time on pH of prepared tablet samples are presented in Table 3 and Figure 4. At room temperature, the pH content of tablet sample decreased from 4.2 to 4 and then 3.8 during storage. The pH levels of the tablet samples indicated that they are slightly acidic.

The effect of storage time on total acidity content of prepared tablet samples

The acidity of natural fruit juices is the result of mainly of their content of organic acids. In this study the total acidity of prepared tablet samples were determined monthly by simple direct titration with 0.1 M sodium hydroxide using phenolphthalein as an indicator. The total acidity of in this sample were not significantly changed during the storage time. The result are showed in Table 3 and Figure 5.

The effect of storage time on vitamin C content of prepared tablet samples

Vitamin C is an oxidant and in water, it readily oxidizes first to dehydroascorbic acid. Its content in food can decrease during food preparation and storage. In this research, the vitamin C content of fresh sample was 44.88 mg / 100 g. During the period of 3 months, the vitamin C content was reduced at room temperature conditions. At room temperature, the vitamin C content decreased from 44.88 to 35.90 mg/ 100 g, 26.92 to 26.92 mg / 100 g in tablet samples after 1 month, 2 months, 3 months of storage time, respectively. The results are shown in Table 3 and Figure 4.

Table 3 The Effect of Storage Time of Prepared Nutraceutical Tablets

No.	Month	Water Content (%)	Ash Content (%)	pH	Vitamin C (mg/100 g) Content	Total acidity content (%)
1.	0	29.94	4.0	4.2	44.88	2.08
2.	1	28.49	3.9	4.0	35.90	2.03
3.	2	26.59	3.1	3.8	26.92	1.97
4.	3	20.89	3.0	3.8	26.92	1.87

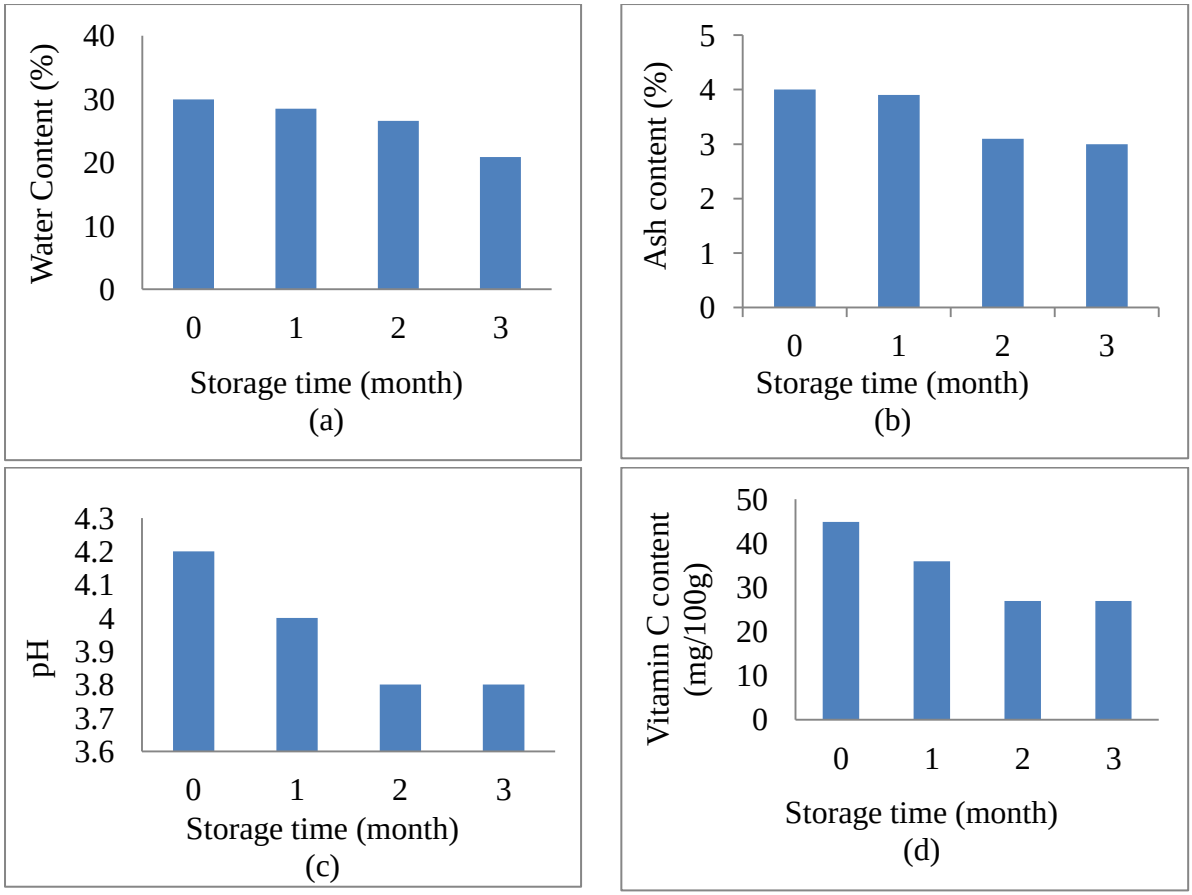


Figure 4 The varation of (a) water content, (b) ash content, (c) pH, (d) vitamin C content of prepared nutraceutical tablets during 3 months storage

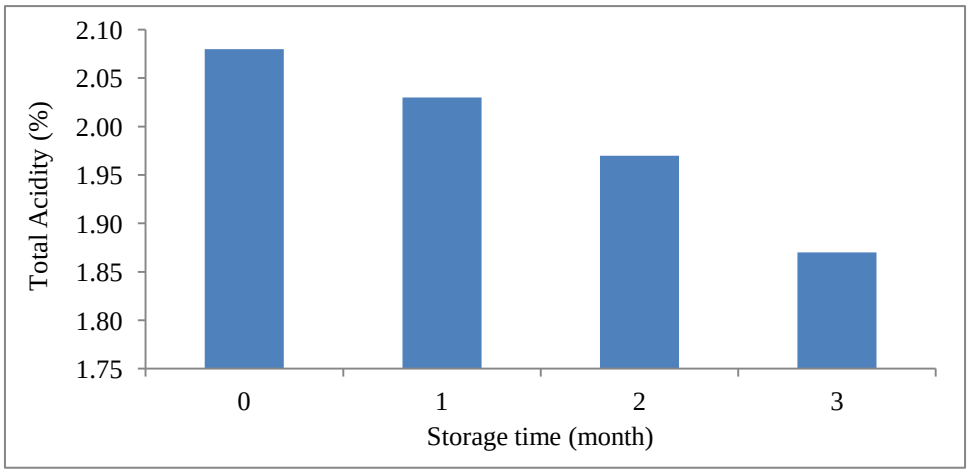


Figure 5 The variation of total acidity contents of prepared nutraceutical tablets during 3 months storage

Mineral Content

Adequate mineral intake is essential throughout the entire life but especially for normal growth and immune function , and to prevent chronic diseases in adulthood (Cabrera-vique *et al.*, 2016).

In the present work the mineral contents found in nutraceutical tablet samples are K, Si, Ca, P, S, Fe, Mn, Rb, Cu, Sr and Zn. From the result the potassium percent was higher than the other metals. The data are shown in Table 4 and Figure 6.

Table 4 Relative Abundance of Elements in the Prepared Nutraceutical Tablets

No	Element	Relative abundance (%)
1.	K	0.549
2.	Si	0.138
3.	Ca	0.118
4.	P	0.024
5.	S	0.021
6.	Fe	0.008
7.	Mn	0.003
8.	Rb	0.002
9.	Cu	0.002
10.	Sr	0.002
11.	Zn	0.001

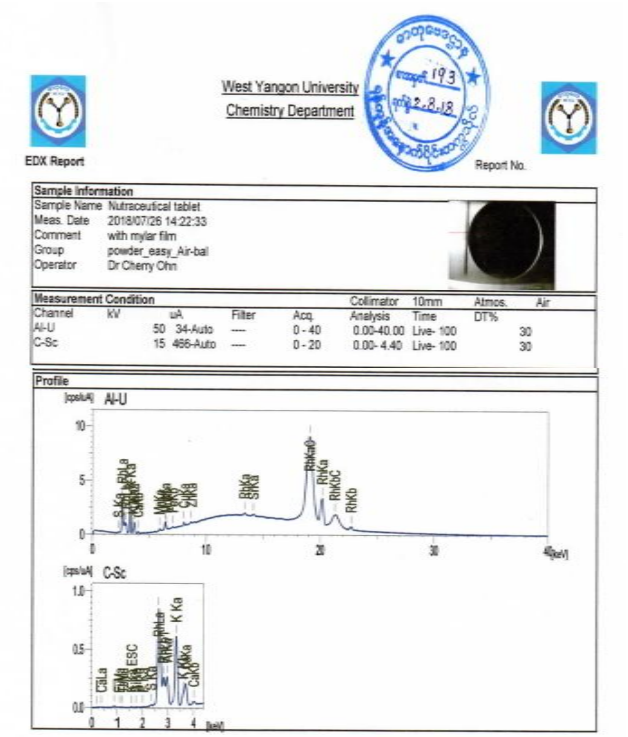


Figure 6 EDXRF spectrum of the prepared nutraceutical tablet

Preliminary Phytochemical Investigations

The phytochemical are naturally occuring substances in medical plants to cure various diseases. The phytochemical screening as qualitative analysis to explore the phytochemicals present in various parts of plants. The medical plants that had been explore are the rich source of natural medicinal agents. The plant leaf, pod and bark of various extracts ethanol, aqueous and ethyl acetate has been identified through phytochemical screening test includes a series of phytochemicals as flavonoids, tannins, polyphenols, reducing sugars, carbohydrates, proteins, saponins, glycosides , steroids terpenoids and α - amino acid (Bhattacharya *et al*., 2015). In the present work it was found that carbohydrates, cyanogenic glycosides, tannins and steroids are absent in the nutraceutical tablet samples. The data are shown in Table 5.

Table 6 Preliminary Phytochemical Investigation of the Prepared Nutraceutical Tablet Samples

No.	Test	Extract	Test Reagents	Observation	Result
1	Alkaloids	1% HCl	Dragendorff's reagent Sodium picrate Wagner's reagent Mayer's reagent	Orange ppt Yellow ppt Brown ppt White ppt	+ + + +
2	Amino-acids	H_2O	Ninhydrin reagent	purple spot on TLC	+
3	Carbohydrates	H_2O	10 % α napthol and H_2SO_4	Red ring	+
4	Cyanogenic Glycosides	H_2O	Sodium picrate solution	No brick red colour ppt	-
5	Flavonoids	EtOH	Mg tunning and conc: HCl	Pink colour solution	+
6	Glycosides	H_2O	10 % lead acetate	White ppt	+
7	Phenolic compounds	EtOH	5 % $FeCl_3$	Dark blue colour solution	+
8	Organic acid	EtOH	Bromocresol green	Yellow colour solution	+
9	Reducing sugars	H_2O	Benedict's Solution	Brick-red colour ppt	+
10	Saponins	H_2O	Distilled water	Forthing	+
11	Starch	H_2O	1 % Iodine	Bluish-black solution	+
12	Steroids	PE	Acetic anhydride & conc: H_2SO_4	No Green colour solution	-
13	Terpenoids	$CHCl_3$	Acetic anhydride & con: H_2SO_4	Pink colour solution	+
14	Tannins	H_2O	1% Gelatin	No Green colour solution	-

(+) = present, (-)= Absent, (ppt) = Precipitate

Antimicrobial Activity

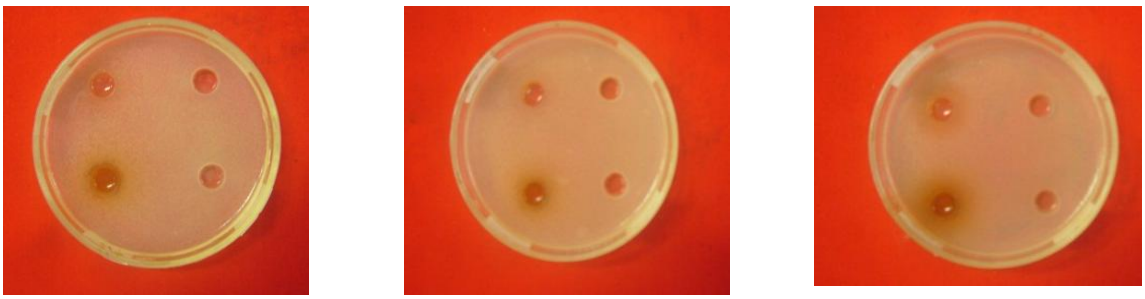
A variety of laboratory methods can be used to evaluate or screen the in vitro antimicrobial activity of an extract or a pure compound. The most known and basic methods are the disk diffusion and broth or agar dilution methods. Other methods are used especially for antifungal testing. In this research, screening of antimicrobial activity of crude extracts has been done by agar well diffusion method, the different extracts from the sample tablets were tested with 6 species of microorganisms (*Bacillus subtilis, S.aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans and Escherichia coli.*). The measurable zone diameter in (mm) is a measure of the degree of antimicrobial activity. The more active extract shows more remarkable zone of inhibition. In Tables 6 and Figure 7 the mean zone diameters of different extracts were found in the range of 11 to 15 mm. It was observed that ethanol extract and water extract were not significantly different in the treatment of disease caused by 6 microorganism species. But

for *B. subtilis*, the water extract is more effective than ethanol extract according to the mean zone diameter of 15 mm and 13 mm. These microorganisms are found in leprosy, skin lesion, carbuncles, pyemia, furunculosis, diarrhea, pneumonia, suppurative lesion, thrush, leukorrhea disease (Mahajan, 2015).

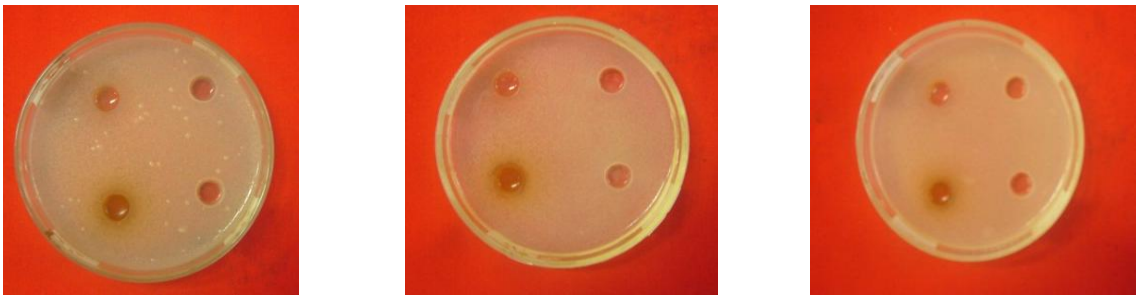
Table 7 Inhibitiion Zone Diameters (mm) of Antimicrobial Activity for Nutraceutical Tablet

Nutraceutical Tablets Extract	Inhibition zone diameter (mm)					
	Gram-positive bacteria			Gram-negative bacteria		Fungi
	B.subtilis	***S. aureus***	***B. pumilus***	***P. aeruginosa***	***E. coli***	***C. albicans***
Ethanol	13 (+)	12 (+)	13 (+)	12 (+)	13 (+)	13 (+)
Watery	15 (++)	13 (+)	14 (+)	13 (+)	14 (+)	14 (+)

Agar well – 10mm
10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)
(-) no activity



(a) Bacillus subtilis *(b) Staphylococcus aureus* *(c)Pseudomonas aeruginosa*



(d) Bacillus pumilus *(e) Candida albicans* *(f) Escherichia coli*

Figure 7 Antimicrobial activity screening of watery and ethanol extracts from prepared nutraceutical tablet samples

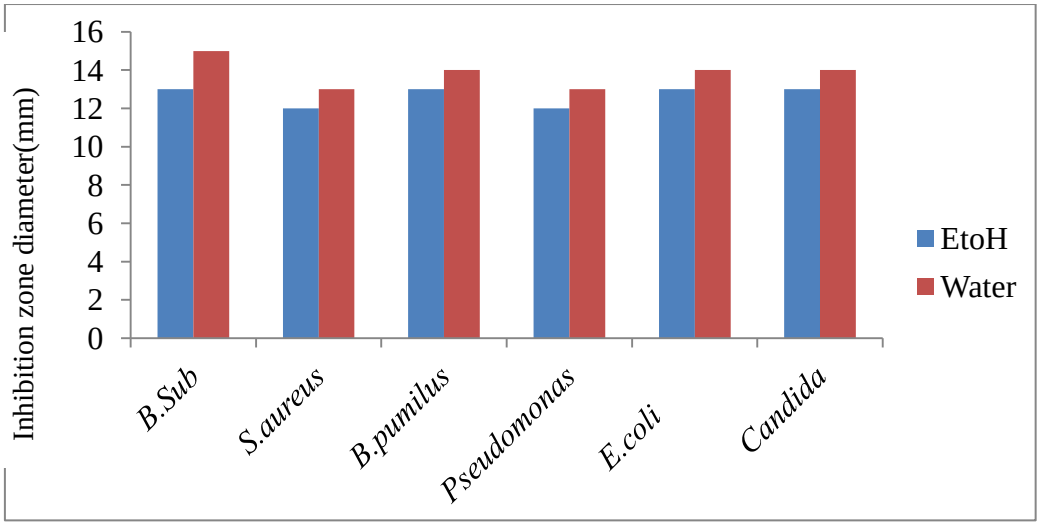


Figure 8 A bar graph of antimicrobial activity of nutraceutical tablet

Cytotoxicity

The cytotoxicity of water extract of nutraceutical tablets was evaluated by brine shrimp cytotoxicity bioassay. This bioassay is general toxicity screening for bioactive plants and their derivatives. A model animal that has been used for this purpose is the brine shrimp, *Artemia salina*. The cytotoxicity of crude watery extract was expressed in term of mean ± SEM (standard error mean) and LD_{50} (50 % Lethality Dose) and the results are shown in Table 8. In this experimental, standard potassium dichromate ($K_2Cr_2O_7$) and caffeine were chosen because $K_2Cr_2O_7$ is well-known toxicity in this assay (Salinas and Fernendez, 2006) and caffeine is a natural product.

As shown in Table 8, the crude watery extract was not cytotoxic to brine shrimp up to maximum dose of 1000 µg / mL. The LD_{50} values of standard $K_2Cr_2O_7$ < 1µg/ mL and caffeine was > 1000 µg / mL.

Table 8 Cytotoxicity Assay of Watery Extract from Nutraceutical Tablet Samples against *Artemia Salina* (Brine Shrimp)

Sample	Dead % of brine shrimp in various contents of sample (µg/L)				LD_{50} (µg/L)
	1	10	100	1000	
$K_2Cr_2O_7$	56.67	83.33	95.00	100	<1
Caffeine	0.00	0.00	10.00	13.33	>1000
Nutraceutical Tablet	13.33	16.67	23.33	43.33	>1000

Conclusion

In this study, from the present preparation of Nutraceutical tablet From selected fruits and study on its nutritional values, mineral contents and their physicochemical properties, the following inferences could be deduced. The nutritional values of the sample were determined by AOAC and Dumas nitrogen analyser. The water content 29.94 %, ash content 4.00 %, protein content 1.89 %, Fiber content 2.22 %, fat content 3.04 %, carbohydrate content 58.91 %, energy value 270.56 kcal/100 g and vitamin C content 44.88 mg / 100 g were observed. The pH of the prepared sample was 4.20 and the total acidity of 2.08 mg /100 g respectively. The reducing sugar content in prepared sample was 1.65 %. The effect of storage time on water content, ash content, vitamin C content, total acidity contents and pH of prepared tablet samples were also determined. From the study work, the water content slightly decreased from 1 month to 3 months storage time duration. The ash content also slightly decreased and the vitamin C content significantly decreased within 3 months duration time. The total acidity were slightly decrease and pH values were not much changed with the longer storage time. The mineral contents of prepared tablet sample were determined by ED XRF spectrum. It was found that 0.549 % K, 0.138 % S, 0.118 % Ca, 0.024 % P, 0.02 % S, 0.008 % Fe, 0.003 % Mn, 0.002 % Rb, 0.002 % Cu, 0.002 % Sr and 0.001 % Zn respectively. Among the presented metals, percent of potassium is higher than other metals. The preliminary phytochemical tests indicated that various types of secondary metabolites such as alkaloids, flavonoids , glycosides , phenolic compounds, reducing sugars, saponins, terpenoids and organic acids together with α-amino acids and starch were present in the sample. The antimicrobial activity of tablet samples from medicinal plant species has been evaluated in vitro against six microorganisms including five bacterial species (*B. subtilis, S. aureus, P. aeruginosa , B.pumilus, E.coli*) and one fungus species (*Candida albicans*). It was observed that, ethanol and watery extracts of tablet samples exhibited antimicrobial activity. The crude watery extract was not cytotoxic to brine shrimp up to maximum dose of 1000 µg / L.

Acknowledgements

The authors would like to thank the Department of Higher Education, Ministry of Education, Yangon, Myanmar, for allowing us to carry out this research programme. Thanks are also extended to the Myanmar Academy of Arts and Science and Professor Dr Ni Ni Than, Head of Department of Chemistry, University of Yangon for allowing to carry out this research programme.

References

Anderson, J. W., Baird, P. and Davis, R. H. (2009). "Health Benefits of Dietary Fiber". *J. of Nutrition*, vol. 67(4), pp. 188- 205

Anindita, D., Sikha, B. and Biswajit, D. (2016). "Pharmacological Activities of Baheda (*Terminalia bellerica*) A review". *J. of Pharmacognosy and Phytochemistry*, vol. 5(1), pp. 194-197

Anwesa, B., Kumar, B.S. and Ranjan, C.R. (2013). "The Development of *Terminalia Chebula Retz*. (Combretaceae) in Clinical Research". *J. of Asian Pacific. Tropikcal Biomedicine*, vol. 3(3), pp. 244-252

AOAC. (2000). *Official Method of Analysis*. Washinton DC: 17th Ed., Association of Official Analytical Chemists , pp. 86-275

Bhattacharya, M., Singh, A. and Khyani, C.R. (2015). "Preliminary Phytochemical Investigation of Various Extracts of Dalbergia sis 00". *J. of Applied Chemistry*, vol. 8 (12), pp. 32-35

Cabrera- Vique, C., Bergillos-Meca, T. and Seiquel, I. (2016). "Mineral content in Fast Foods". *J. of Department of Nutrition and Food Science, university of Granada*, Spain.

Mahajan, D. and Jain, S. (2015) . "Antimicrobial analysis of Triphala and comparison with its individual constituents". *Indian J. Pharm. Biol. Res.*, vol. 3(3), pp. 55-60

Nielsen, S. (2014). *Introduction to Food Analysis*. New York: 4th Ed., Dordrecht Heidelberg, pp. 1-352

Ozkan, M., Kirca, A. and Cemeroglu, B. (2004). "Effects of Hydrogen Peroxide on the Stability of Ascorbic Acid During Storage in Various Fruit Juices". *J. of Food Chemistry*, vol. 88(4), pp. 591-597

Rohit, S., Thakur, G. S., Sanodiyi, S. S., Sanodiyi, A., Savita, A., Pandey, M., Sharmal, A. and Bisen, P. S. (2012). "Therapeutic Potential of Calotropisprocera: A giant milk weed. IOSR". *J. of Pharmacy and Biological Sciences* (IOSR-JPBS) ISSN: 2278-3008, **4** (2), 42-57

Salinas, M. M. G. and Fernandez, S. S. (2006). "A Modified Microplate Cytotoxicity Assay with Brine Shrimp Larvae (*Artemia salina*)". *Pharmacology*, vol. 3, pp. 633-638

Swaroopa, G. and Srinath, D. (2017). "Nutraceuticals and their Health Benefits". *Int.J.Pure App. Biosic*, vol. 5(4), pp. 1151-1155

William, L. (1993). "Protein Fructosylation Fructose and the Maillard Reaction". *The American Journal of Clinical Nutrition American society for Nutrition*, vol. 58 (5), pp.7795-7787