ANTIMICROBIAL ACTIVITY OF ISOLATED PORPHYRIN DERIVATIVE COMPOUNDAND STUDY ON THE CYTOTOXICITY, ANTIOXIDANT ACTIVITY OF CRUDE EXTRACTS OF THE WHOLE PLANT OF *Corallodiscus lanuginosus* (Wall. ex R.Br.)Burtt

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Abstract

In this research paper, *Corallodiscus lanuginosus* (Wall. ex R.Br.)Burttwhich is one of the Myanmar indigenous medicinal plants known as Pan ma o` was selected for the study on the cytotoxicity and antioxidant activity. The cytotoxicity of the ethanol extract of the selected sample was more toxic to brine shrimp than the water extract. The LD_{50} values of ethanol and water extracts were 380μg/mL and 423μg/mL, respectively. On the other hand, the EtOH and water extracts were cytotoxic to brine shrimp up to maximum dose of 1000 μg/mL. The LD_{50} values of standard $K_2Cr_2O_7$ and caffeine are 265 μg/mL and >1000 μg/mL, respectively. In addition, the larger radical scavenging activity to scavenge DPPH radical was observed in ethanol extract, which inhibited 50% of free radicals at the concentration (IC_{50}) of 7.10 μg/mL than the water extract which inhibited 50% of free radicals at the concentration (IC_{50}) of 11.13 μg/mL. Moreover, antimicrobial activities of the isolated porphyrin derivative compound were examined by using agar well diffusion method. The ethyl acetate extract of this porphyrin derivative compound responds high activities on *Bacillus subtilis, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans and Escherichia coli.*

Keywords: Pan ma o`, porphyrin, cytotoxicity, antioxidant activity, antimicrobial activity

Introduction

The knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures are used by traditional medicine in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness" (WHO, 2010).There are many different systems of traditional medicine, and the philosophy and practices of each are influenced by the prevailing conditions, environment, and geographic

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area within which it first evolved (WHO, 2005), however, a common philosophy is a holistic approach to life, equilibrium of the mind, body, and the environment, and an emphasis on health rather than on disease. Generally, the focus is on the overall condition of the individual, rather than on the particular ailment or disease from which the patient is suffering, and the use of herbs is a core part of all systems of traditional medicine (Engebretson, 2002; Conboy *et al.*, 2007; Rishton 2008; Schmidt *et al.*, 2008).Over the past 100 years, the development and mass production of chemically synthesized drugs have revolutionized health care in most parts of the word. However, large sections of the population in developing countries still rely on traditional practitioners and herbal medicines for their primary care. In Africa up to 90% and in India 70% of the population depend on traditional medicine to help meet their health care needs. In China, traditional medicine accounts for around 40% of all health care delivered and more than 90% of general hospitals in China have units for traditional medicine (WHO, 2005). However, use of traditional medicine is not limited to developing countries, and during the past two decades public interest in natural therapies has increased greatly in industrialized countries, with expanding use of ethnobotanicals. In the United States, in 2007, about 38% of adults and 12% of children were using some form of traditional medicine (Ernst *et al*., 2005; Barnes *et al.*, 2008).

Materials and Methods

Determination of Cytotoxicity of Crude Extracts by Brine Shrimp Lethality Bioassay

Cytotoxicity of crude extracts of selected sample was investigated by brine shrimp lethality bioassay according to the procedure described by Dockery and Tomkins (2000).

Preparation of solutions

(a) Preparation of sample solutions

Each crude extract (5 mg) was dissolved in 5 mL of distilled water to obtain stock solution (1000 μg/mL). Desired concentrations (1000, 500, 250, 125, 62.5, 31.25 and 15.125 μg/mL) of each crude extract were prepared from this stock solution by two fold serial dilution with distilled water.

(b) Preparation of standard solutions (Potassium dichromate and caffeine)

Each of potassium dichromate (5 mg) and caffeine (5 mg) was dissolved in 5 mL of distilled water. These stock solutions were two fold serially diluted with distilled water to get the standard solutions with the concentrations of 1000, 500, 250, 125, 62.5, 31.25 and 15.125 µg/mL.

(c) Preparation of artificial sea water

Artificial sea water [3.8% (w/v) NaCl] was prepared by dissolving (38 g) of sodium chloride in 1 L of distilled water.

Hatching of brine shrimp

The brine shrimp (*Artemiasalina*) was used in this study for cytotoxicity bioassay (Ali *et al*., 2013). Brine shrimp cysts were purchased from pet shop, Baho Road, Hlaing Township, Yangon Region.

Brine shrimp cysts (0.5 g) were added to the 1.5 L of artificial sea water bottle. The suspension was aerated by bubbling air into the funnel and kept for 24 h at room temperature.

Procedure

After aeration had been removed, the suspension was kept for 1 h undisturbed, whereby the remaining unhatched eggs dropped. In order to get a higher density of larvae, one side of the separating funnel was covered with aluminium foil and the other illuminated with a lamp, whereby the phototropic larvae were gathering at the illuminated side and could be collected by pipette. The shrimp larvae were transferred to an agar well filled with 9 mL of salt water and the dead larvae counted (number N). A solution of crude extract (31.25 - 1000 ppm) (1 mL reach) was added and the plate kept at room temperature in the dark. After 24 h, the dead larvae were counted in each well under the microscope (number A). The still living larvae were killed by addition of ca. 0.5 mL methanol so that subsequently the total number of the animals could be determined (number G). The mortality rate M was calculated in percent. Each test row was accompanied by a brine solution (number B). The mortality rate M was calculated by using the following formula:

$$M = \left[\frac{(A - B - N)}{(G - N)} \right] \times 100$$

M = percent of the dead larvae after 24 h

A = number of the dead larvae after 24 h
B = average number of the dead larvae in the brine solution after 24 h
N = number of the dead larvae before starting of the test
G = total number of brine shrimps

The control solution was prepared as the above procedure by using distilled water instead of sample solution. The results are summarized in Table1.

Determination of Antioxidant Activity of Crude Extracts by DPPH Free Radical Scavenging Assay

The free radical scavenging activity of crude extracts (ethanol and water extracts) of Pan ma o` was measured by using DPPH free radical scavenging assay (Marinova and Batchvarov, 2011).

Preparation of solutions

(a) Preparation of 0.002% (w/v) DPPH solution

0.002% DPPH solution was prepared in the brown coloured bottle by dissolving 2 mg of DPPH powder in 100 mL of ethanol. It must be stored in the refrigerator for no longer than 24 h.

(b) Preparation of standard solutions (Gallic acid)

The stock solution (200 μg/mL) of standard gallic acid was prepared by dissolving (2 mg) of each compound in 10 mL of ethanol. The stock solutions were two fold serially diluted with ethanol to get the standard solutions with the concentrations of 200, 100, 50, 25, 12.5, 6.25 and 3.125 μg/mL.

(c) Preparation of test sample solutions

The stock solution (200 μg/mL) of the crude extract was prepared by dissolving (2 mg) of respective crude extract in 10 mL of ethanol. The stock

solutions were two fold serially diluted with ethanol to get the sample solution with the concentrations of 200, 100, 50, 25, 12.5, 6.25 and 3.125 μg/mL.

(d) Preparation of blank solution

Blank solution was prepared by mixing 1.5 mL of sample solution with 1.5 mL of ethanol.

Procedure

DPPH radical scavenging activity was determined by UV-visible spectrophotometer (Marinova and Batchvarov, 2011).

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 incubated at room temperature were shaken on shaker for 30 min. After 30 min, the absorbance values of these solutions were measured at 517 nm and the percentage of radical scavenging activity (% RSA) was calculated by the following equation.

$$\% \text{ RSA} = [\{(A_{DPPH} - A_{Sample}) - A_{Blank}\}/ A_{DPPH}] \times 100$$

where, % RSA = % radical scavenging activity of test sample

A_{DPPH} = absorbance of DPPH in ethanol solution

A_{Sample} = absorbance of sample + DPPH solution

A_{Blank} = absorbance of sample + EtOH solution

The antioxidant activity (IC_{50}) is expressed as the test substance concentration (μg/mL) that results in a 50% reduction of initial absorbance of DPPH solution. IC_{50}(50% inhibition concentration) values were calculated by linear regressive excel program. The standard deviation was also calculated by the following equation.

$$\text{Standard Deviation (SD)} = \sqrt{\frac{(\bar{x} - x_1)^2 + (\bar{x} - x_2)^2 + \ldots (\bar{x} - x_n)^2}{(n-1)}}$$

Results and Discussion

Cytotoxicity of Crude Extracts

The cytotoxicity of water and ethanol extracts ofselected sample were 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, *Artemiasalina* (Tawaha, 2006).

The cytotoxicity of crude extracts were expressed in term of mean ± SEM (standard error mean) and LD_{50} (50 % Lethality Dose) and the results are shown in Table 1 and Figure 1. In this experiment, standard potassium dichromate ($K_2Cr_2O_7$) and caffeine were chosen because $K_2Cr_2O_7$was well-known for its toxicity in this assay (Salinas andFernandez, 2006) and caffeine is a natural product.

As shown in Table 1, the cytotoxicity of the ethanol extract of the selected sample was more toxic to brine shrimp than the water extract. The LD_{50} values of water and ethanol extracts were 423μg/mL and 380 μg/mL respectively. The water and ethanol extracts were cytotoxic to brine shrimp up to maximum dose of 1000 μg/mL. The LD_{50} values of standard $K_2Cr_2O_7$ and caffeine are 265μg/mL and >1000 μg/mL, respectively.

Table 1: Cytotoxicity of Ethanol and Water Crude Extracts

Samples	**Percent (%) of the dead larvae at different concentrations of the samples after 24 h**							**LD_{50} (□g/mL)**
	15.625	**31.25**	**62.5**	**125**	**250**	**500**	**1000**	
Water Extract	5.56	13.79	20.00	27.27	40.00	54.55	58.06	423
EtOH Extract	12.75	17.65	39.29	34.48	35.29	63.89	70.00	380
*$K_2Cr_2O_7$	17.50	23.53	35.48	42.31	49.02	69.23	74.47	265
*Caffeine	3.19	10.81	11.43	23.53	29.03	30.43	37.14	>1000

*****standard

These results revealed that the plant extracts possess cytotoxic activity and can be the sources of cytotoxic compounds. The selected plant sample can

be used in traditional medicine to treat many kinds of diseases. The reported cytotoxic plants in this study are worth of further pharmacological and phytochemical studies in order to define what kind of bioactivity they have and to isolate the natural active constituent, which are responsible for the activity.

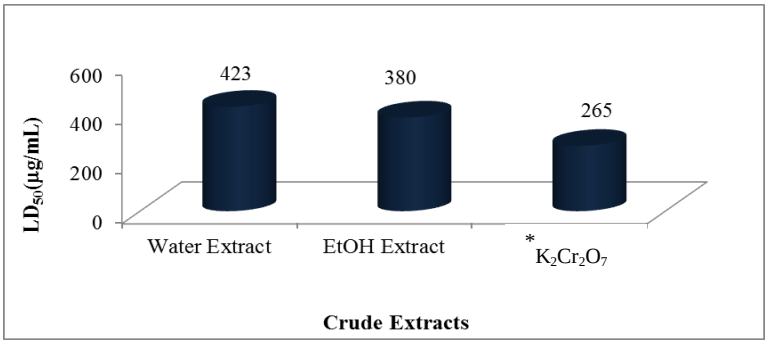

Figure 1: A bar graph representing LD_{50} values of watery and ethanol extracts of the selected sample and standard $K_2Cr_2O_7$

Antioxidant Activity of Crude Extracts by DPPH Free Radical Scavenging Assay

Most of the medicinal plants possess phytochemicals and antioxidant activity. Flavonoids and tannins are phenolics which are a major group of compound in plants. These compounds act as primary antioxidant or free radical scavengers (Ayoola *et al*., 2008). The antioxidant activity of water and ethanol extracts of selected sample were studied by DPPH free radical scavenging assay (Marinova and Batchvarov, 2011). Gallic acid was used as standard.

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay is widely used to investigate the scavenging activities of several natural compounds such as crude extracts of plants. DPPH radical is scavenged by antioxidant through the donation of electron forming the reduced DPPH. Sample's colouris changed from purple to pale yellow which can be quantified by its decrease in absorbance at wavelength 517 nm (Maw *et al*., 2011). The radical scavenging activity of crude extracts was expressed in terms of % RSA (Table 2 and Figure 2) and IC_{50}(50 % inhibition concentration) (Table 3 and Figure 3).

From these observations, the larger radical scavenging activity to scavenge DPPH radical was observed in ethanol extract, which inhibited 50% of free radicals at the concentration (IC_{50}) of 7.10µg/mL than the water extract which inhibited 50% of free radicals at the concentration (IC_{50}) of 11.13µg/mL. These results were found to be slightly higher than standard gallic acid (IC_{50}= 3.79µg/mL).

It can be concluded that the antioxidant potency of the ethanol extract was found to be stronger than that of the water extract, but weaker than the standard gallic acid.

Table 2: Radical Scavenging Activity of Crude Extracts

Samples	% RSA ± SD at Different Concentrations (µg/mL)						
	3.125	**6.25**	**12.5**	**25**	**50**	**100**	**200**
Ethanol extract	36.58	48.96	56.69	71.38	79.27	84.15	91.49
Water extract	16.47	18.02	59.78	75.64	87.32	89.48	94.20
Gallic acid	47.95	57.64	62.40	67.00	77.83	92.61	96.06

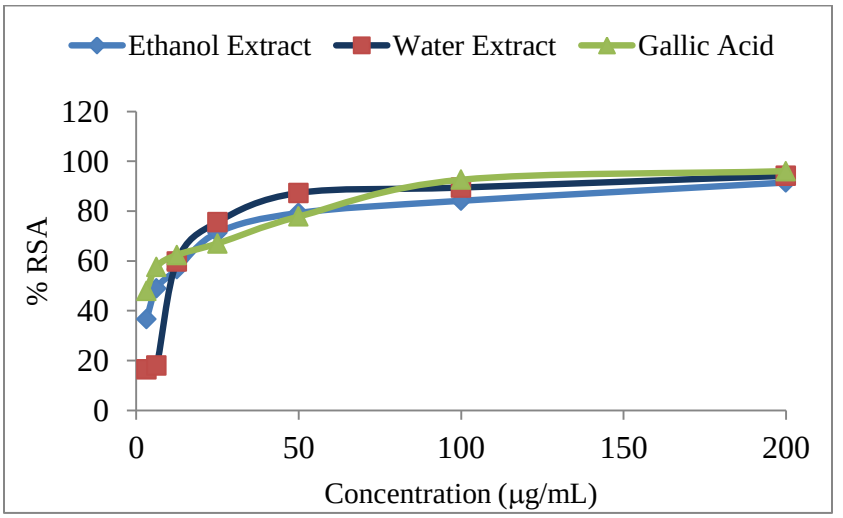

Figure 2: % RSA of crude extracts comparison with standard gallic acid

Table 3: IC_{50} of Crude Extracts and Gallic Acid

No.	Sample	IC_{50} (µg/mL)
1	EtOH extract	7.10
1	EtOH extract	7.10
1	EtOH extract	7.10

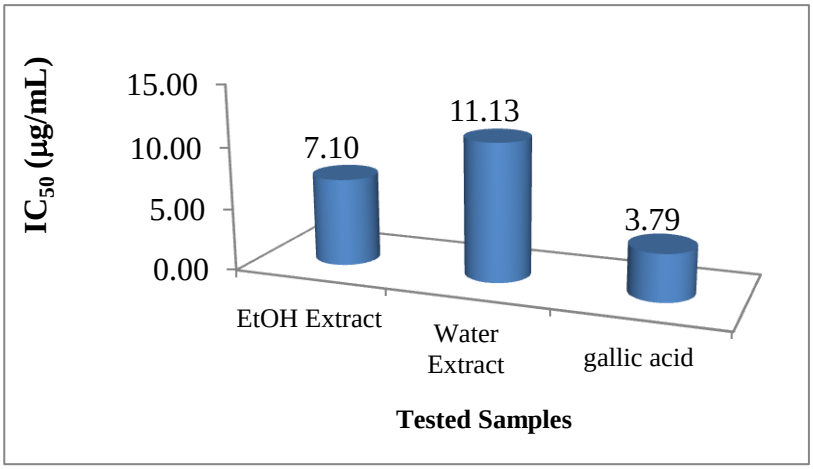

Figure 3: IC_{50} values of crude extracts and gallic acid

Antimicrobial Activities of the Porphyrin Derivative Compound

Porphyrin derivative compound could be isolated from the whole plant of Pan ma o` in January 2017 at Department of Chemistry, Mandalay University (Thinn Myat New *et al.*, 2017). It had been reported in Myanmar Academy of Arts and Science, 2017 (Thinn Myat Nwe and Myo Myo, 2017). Antimicrobial activities of isolated compound were checked by using agar well diffusion method on six selected organisms. The results are described in Table 4 and Figure 4.

Table 4: Results of Antimicrobial Activities of Porphyrin Derivative Compound

Sample	Solvent	Organisms and Inhibition Zone					
		I	**II**	**III**	**IV**	**V**	**VI**
Pure compound	EtOAc	+++ 29.11	–	+++ 27.45	+++ 28.03	+++ 27.13	+++ 29.85
Control	EtOAc	–	–	–	–	–	–

agar well ~ 10mm
10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)

Organisms
I *Bacillussubtilis*
II *Staphylococcus aureus*
III *Pseudomonousaeruginosa*
IV *Bacillus pumilus*
V *Candida albicans*
VI *E. coli*

According to this table, the ethyl acetate extract of isolated compound gives rise to high activities on all selected organisms except *Staphylococcus aureus*.

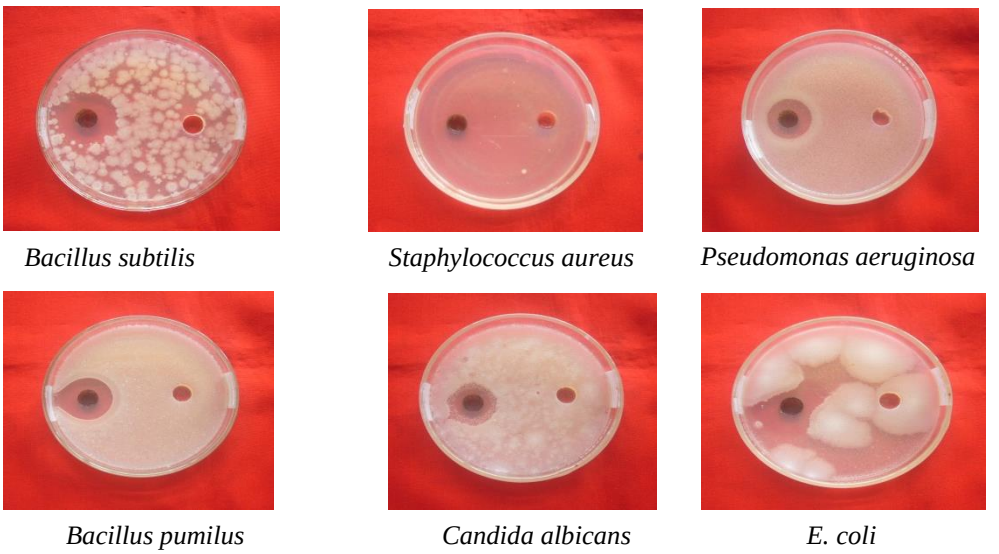

Figure 4: Antimicrobial activities of porphyrin derivative compound

Conclusion

In this research work, one of Myanmar indigenous medicinal plants, locally known as Pan ma o` was selected for the examination of cytotoxicity, antioxidant activity andthe determination of antimicrobial activities of isolated porphyrin derivative compound.

The antimicrobial activities of isolated porphyrin derivative compound give rise to high activities on all selected organisms except *Staphylococcus aureus*. The cytotoxicity of the ethanol extract of the sample was more toxic to brine shrimp than the water extract. The LD_{50} values of ethanol and water extracts were 380μg/mL and 423 μg/mL, respectively. In cytotoxicity, both extracts were lower than $K_2Cr_2O_7$ (LD_{50} = 265 μg/mL), higher than caffeine (LD_{50}>1000 μg/mL). In addition, the larger radical scavenging activity to scavenge DPPH radical was observed in ethanol extract, which inhibited 50% of free radicals at the concentration (IC_{50}) of 7.10 μg/mL than the water extract which inhibited 50% of free radicals at the concentration (IC_{50}) of 11.13 μg/mL.

Acknowledgements

The authors would like to thank the MyanmarAcademy of Arts & Science for allowing to present this paper, Professor and Head, Dr Daw Khin Aye Tint Nwe and Professor Dr Khin Mar Yee, Department of Chemistry, Mandalay University of Distance Education, Myanmar, for their valuable advice and permission. The author is also thankful to Dr Khine Zar Wynn Lae, Lecturer, Department of Chemistry, YangonUniversity for her providing to complete this research work.

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