SCREENING OF SOME BIOLOGICAL ACTIVITIES FROM LEAVES AND FLOWERS OF *Melastoma malabathricum* L. (NYAUNG-YE-O-PAN)

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Abstract

The present study concerned with the determination of phytochemical constituents, nutritional values and biological activities such as antimicrobial, antioxidant, total phenol and flavonoid contents of leaves and flowers of *Melastoma malabathricum* L. (Nyaung-ye-o-pan, NYOP). The preliminary phytochemical results showed the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins and terpenoids in both leaves and flowers of NYOP. Some nutritional values such as the moisture contents (6.00 % and 8.00 %), the ash contents (7.53 % and 4.00 %), the fiber contents (13.81 % and 11.17 %), the fat contents (4.16 % and 2.44 %), the protein contents (4.55 % and 3.47 %), and the carbohydrate contents (63.95 % and 70.92 %) of leaves and flowers of NYOP were determined by using the respective methods. The screening of antimicrobial activity of crude extracts such as PE, EtOAc, EtOH and H_2O indicated that antimicrobial activity with inhibition zone diameters ranged between 13 mm~25 mm in NYOP (L) and 13 mm~27 mm in NYOP (F). The NYOP (F) showed higher activity than NYOP (L). The antioxidant activity of ethanol and watery crude extracts of Nyaung-ye-o-pan leaves and flowers was investigated by DPPH free radical scavenging assay. The IC_{50} values of NYOP (L) watery and ethanol crude extracts were17.14 µg/mL and 15.91 µg/mL, respectively, and those of NYOP (F) watery and ethanol extracts were 35.39 µg/mL and 21.95 µg/mL. Since the lower IC_{50} values, the higher antioxidant activity of the samples occurs. Thus, the ethanol extract of NYOP (L) was greater antioxidant activity than that of NYOP (F) whereas watery extract of NYOP (F) was greater than that of NYOP (L). The total phenolic contents in watery and ethanol extracts of NYOP (L) and (F) were found to be 416.2 and 212.2; 320.7 and 195.9 mg of GAE/g of extract respectively. Among these, watery extract of NYOP (L) contained the highest phenolic content. The total flavonoid contents in watery and ethanol extracts of NYOP (L) and NYOP (F) were 88.9 and 114.4; 125.6 and 112.2 mg of QE/g of extract respectively. Among these, watery extract of NYOP (F) has highest flavonoid content.

Keywords: *Melastoma malabathricum* L., phytochemical constituents, antimicrobial activity, antioxidant activity, phenolic content, flavonoid content

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Introduction

Traditional medicinal plants contain various constituents which are very useful in both preventive and curative traditional medicine preparations for human beings. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs and antimicrobial drugs(Munin, 2011).

In this study, our attention has been focused on *Melastoma malabathricum* L. that belongs to the family Melastomataceae and it is called Nyaung-ye-o-pan (NYOP) in Myanmar. It is native to India, China, Japan, Cambodia, Myanmar, Malaysia, Nepal, Philippines, Thailand and Vietnam (Nadkarni, 2000). It is an evergreen erect shrub and its average height is 1.5 to 5 m tall but may occasionally grow up to 5 m long. The stems are 4-sided to subterete, generally bristle, covered with small scales and reddish (Zakaria and Mohd, 1994). The leaves are 7 to 12 cm long, slightly rough and hairy on both surfaces. The flowers grow in 5 to 10 clusters and 5 petals. On rare occasions, *M.malabathricum* consists of 3 varieties, dark-purple petals, light pink-magenta petals and (the rare variety) white petals (Susanti *et al*., 2007). The fruits are classified as berries and when they are ripe, they split irregularly to reveal the soft, dark purple, sweet but rather astringent-tasting pulp and numerous orange seeds. The seeds are tasteless and can be eaten and they stain the tongue black (Koya, 2008). The young leaves are to treat diarrhea while the young premature leaves are consumed raw to cure dysentery. Other medicinally usesare to treat ulcers, gastric ulcer, scar, pimple and black spot at skin (Lohezic-Le Devehat *et al*., 2002). The flowers of *M.malabathricum* are also used as a nervous sedative and for hemorrhoidal bleeding. The leaves and flowers are useful for the treatment of cholera, diarrhoea, prolong fever, dysentery, leucohorrea, wounds and skin diseases and for the preparation of gargle (Perry, 1980).

Materials and Methods

Plant materials

The leaves and flowers of NYOP were collected from Hlar-ka-myin Village, Hpa-an Township in Kayin State. The plant was identifiedby the authorized botanist, at Botany Department, Hpa-an University, Myanmar. After cleaning and drying at room temperature, each of the dried samples was ground into powder and stored separately in air-tight containers. Each powder sample was used for determination of nutritional values and phytochemical constituents. The various crude extracts leaves and flowers of NYOP were used for some pharmacological activities such as antimicrobial and antioxidant activity. Each crude extract was used to determine total phenolic and total flavonoid contents.

Chemicals

Ferric chloride, potassium iodide, picric acid, sodium hydroxide, ninhydrin, α-napthol, sulphuric acid, lead acetate, acetic anhydride, iodine, bromocresol green, gelatin, ethanol, magnesium ribbon, hydrochloric acid, chloroform, petroleum ether, meat extract, peptone, sodium chloride, agar powder, DPPH (1,1 diphenyl-2-picrylhydrazyl), vitamin C, Folin-Ciocalteu reagent, sodium carbonate, standard gallic acid, aluminium chloride, potassium acetate and standard quercetin were used.

Instruments

Soxhlet extractor, water bath, Micro-Kjeldahl distillation apparatus, UV-spectrophotometer (UV- 7504 KWF, China), shaker and electric balance were required.

Phytochemical Investigation

The dried powdered samples were used for the chemical tests on the phytochemicalsby using standard procedure (M-Tin Wa, 1972; Trease and Evans, 1980; Shriner *et al*., 1980; Harborne, 1984; Marini-Bettolo *et al*., 1981; Robinson, 1983; Vogel, 1966).

Determination of Nutritional Values

Some nutritional values such as moisture, ash, fiber, fat, protein and carbohydrate contents were made by reported methods (Joslyn, 1970; Pearson, 1976; Steyermart, 1961; Anderson, 1984). The total energy value was determined from the sum of fat, protein and carbohydrate contents.

Screening of Antimicrobial Activity

The screening of antimicrobial activity of various crude extracts such as PE, EtOAc, EtOH and watery extracts of NYOP (L) and NYOP (F) were carried out by agar dics diffusion method at Pharmaceutical Research Department, Ministry of Industry, Yangon, Myanmar. Six microorganisms namely *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli* were used for this test.

Screening of Antioxidant Activity

The antioxidant activity of each crude extract of NYOP leaves and flowers were screened by using DPPH Free Radical Scavenging Assay (Marinova and Batchvarov, 2011). 2 mg of DPPH powder was freshly prepared by dissolving in 100 mL of ethanol. Standard vitamin C was dissolved in 10 mL of ethanol to get the stock solution. Each respective crude extract of NYOP leaves and flowers (2 mg) was dissolved in 10 mL of ethanol to get the stock solution. Each stock solution of standard vitamin C and sample solution were two fold serially diluted with ethanol to get their respective solutions with the concentration of 200, 100, 50, 25, 12.5 and 1.625μg/mL. The blank solution was also prepared by mixing the sample solution 1.5 mL with ethanol. The control solution was prepared by mixing with 1.5 mL of DPPH solution and 1.5 mL of ethanol in brown bottles. These bottles are incubated at room temperature and were shaken on shaker for 30 min. After 30 min, the absorbance values of these solutions were measured at 517 nm.

Determination of Total Phenol Contents

The total phenol content in each sample was estimated by Folin-Ciocalteu method. Each extract (10 mg) was mixed with 10 mL of distilled water. Each extract solution (1 mL) was mixed with 5 mL of FCR solution and incubated for 5 min. To each tube, 4 mL of 1M sodium carbonate solution was added and the tubes were kept in room temperature for 2 h. The absorbance was measured spectrophotometrically at 765 nm. The concentrations of gallic acid equivalent of each of the plant extract were calculated by using linear regression equation from the standard curve of gallic acid. Total phenol content in the plant samples were expressed as mg gallic acid equivalent per 1 g dry plant extracts (Basma *et al*., 2011; Kaur and Poonam, 2014).

Determination of Total Flavonoid Contents

The total flavonoid content in each sample was estimated by Aluminium Chloride Calorimetric Assay. Each extract (10 mg) was mixed with 10 mL of distilled water. Each of this extract solution (0.5 mL) was mixed with 1.5 mL of methanol, 0.1 mL of 1 % $AlCl_3$ solution, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The resultant mixture was allowed to stand for 30 minutes at room temperature. Absorbance of resulting blue solution was measured at 415 nm using spectrophotometrically (KWF UV-7504). In this method quercetin was used as a standard. The concentrations of quercetin equivalent (QE) in plant extracts were calculated by using the linear regression equation from standard calibration curve of quercetin. Total flavonoid content in the plant samples were expressed as mg quercetin equivalent per 1 g dry plant extracts (Basma *et al*., 2011; Kaur and Poonam, 2014).

Results and Discussion

Preliminary Phytochemical Investigation

According to the phytochemical test results, alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins, and terpenoids were present in both leaves and flowers of NYOP. However, cyanogenic glycosides were absent in both of these samples.

Investigation of some Nutritional Values

The moisture content was determined by oven drying method. The moisture contents of NYOP were found to be 6.00 % in leaves and 8.00 % in flowers. Since the moisture content is less than 12 %, the changes for the growth of microorganisms are greatly minimized. The ash content in leaves (7.53 %) was greater than that in flowers (4.00 %). The fiber content in leaves (13.81 %) was also higher than that in flowers (11.17 %). Moreover, the fat and protein contents of leaves of NYOP (4.16 % and 4.55 %) were much higher than that in flowers of NYOP (2.44 % and 3.47 %). However, the carbohydrate contents of leaves (63.95 %) are greatly less than that of flowers (70.92 %). The energy values were found to be (311.44 kcal/100 g) in leaves of NYOP which were also lesser than in flowers of NYOP (319.52 kcal/ 100 g). The results are shown in Table 1 and Figure 1.

Table 1: Some Nutritional Values of Leaves and Flowers of *M. malabathricum* (Nyaung-ye-o-pan)

No.	Nutritional parameters	NYOP (L)	NYOP (F)
1	Moisture (%)	6.00	8.00
2	Ash (%)	7.53	4.00
3	Fiber (%)	13.81	11.17
4	Fat (%)	4.16	2.44
5	Protein (%)	4.55	3.47
6	Carbohydrate (%)	63.95	70.92
7	Energy values (kcal/ 100 g)	311.44	319.52

*base on dry sample

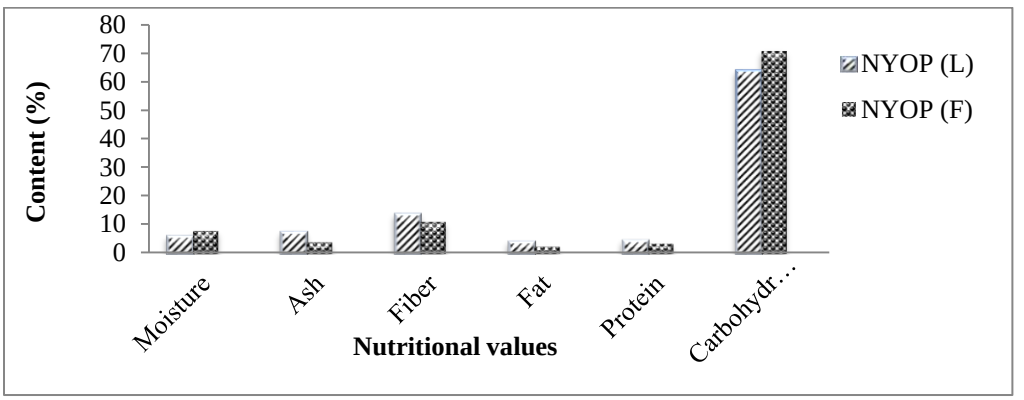

Figure 1: A bar graph diagram showing some nutritional values of leaves and flowers of *M. malabathricum* (Nyaung-ye-o-pan)

Screening of Antimicrobial Activity

Four crude extracts of such as PE, EtOAc, EtOH and H_2O were screened for antimicrobial activity against six different pathogenic microbes using agar well diffusion method. Larger the zone diameter, the more activity is on the test bacteria. According to the results in Figure 2 (a and b) and Table 2, PE and watery extracts of NYOP (L) did not show any antimicrobial activity against all of the microorganism tested. But PE extract of NYOP (F) showed antimicrobial activity except *Bacillus subtilis* and *Escherichia coli*. EtOAc and EtOH crude extracts of NYOP (L) showed antimicrobial activity against all the tested pathogenic microbes. Antimicrobial activity with inhibition zone diameters ranged between 13 mm~25 mm in NYOP (L) and 13 mm~27 mm in NYOP (F). The NYOP (F) showed higher activity than NYOP (L).

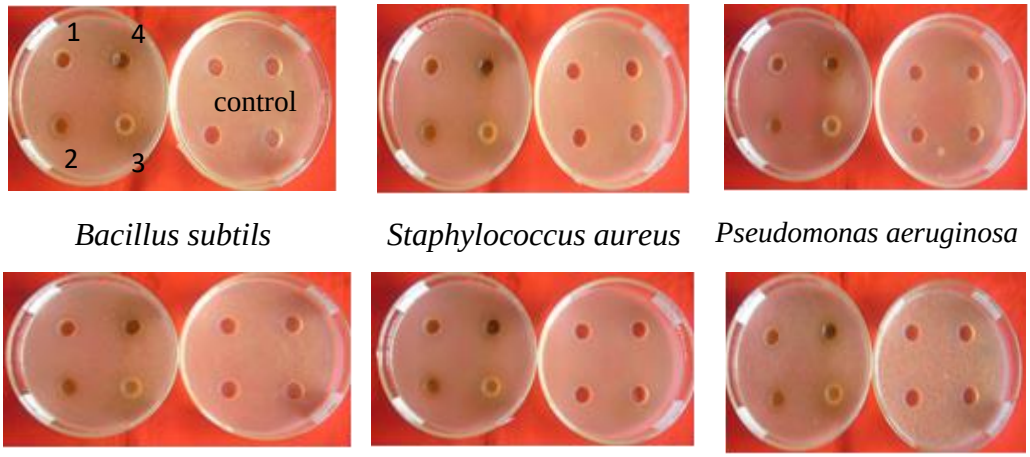

Figure 2(a): Screening of antimicrobial activities of varioucrude extracts from NYOP (L)

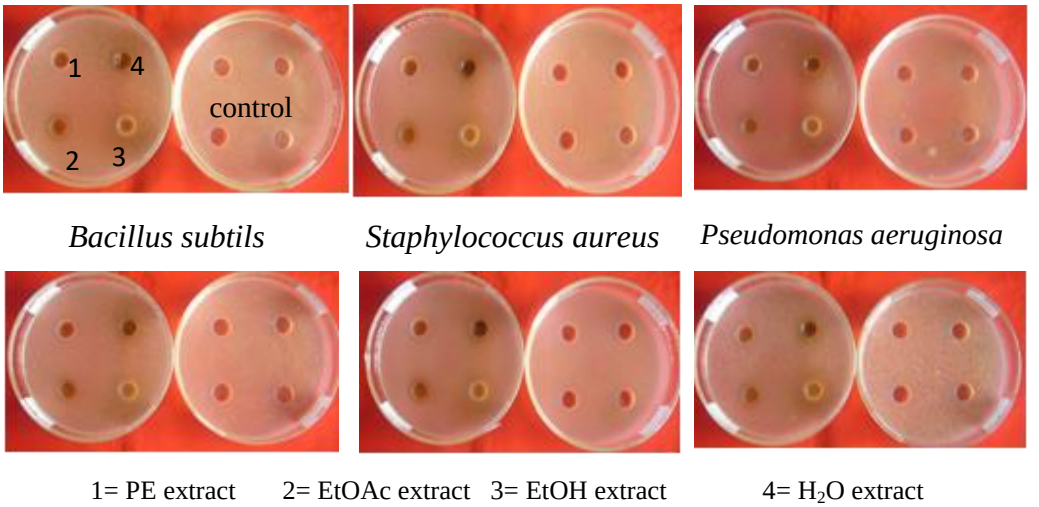

Figure 2(b): Screening of antimicrobial activities of various crude extracts from NYOP (F)

Table 2: Inhibition Zone Diameters of Various Crudes Extracts of Leaves and Flowers of NYOP against Six Microorganisms by Agar Well Diffusion Method

Samples	Solvents	Inhibition Zone Diameter (mm)					
		B. subtilis	***S. aureus***	***P. aeruginosa***	***B. pumilus***	***C. albicans***	***E. coli***
NYOP (Leaves)	PE	-	-	-	-	-	-
	EtOAc	15 (++)	15 (++)	25 (+++)	15 (++)	16 (++)	16 (++)
	EtOH	18 (++)	18 (++)	13 (+)	16 (++)	18 (++)	18 (++)
	H_2O	-	-	-	-	-	-
NYOP (Flowers)	PE	-	13 (+)	15 (++)	13 (+)	13 (+)	-
	EtOAc	15 (++)	15 (++)	20 (+++)	13 (+)	15 (++)	14 (+)
	EtOH	24 (+++)	24 (+++)	27 (+++)	26 (+++)	27 (+++)	25 (+++)
	H_2O	19 (++)	20 (+++)	20 (+++)	18 (++)	20 (+++)	20 (+++)

Diameter of agar well = 10 mm No activity = (-)
10 mm ~ 14 mm = (+) 15 mm ~ 19 mm = (++) 20 mm above = (+++)

Antioxidant Activity of some Crude Extracts from Leaves and Flowers of NYOP by DPPH Free Radical Scavenging Assay

The antioxidant activity was measured in terms of hydrogen donating or radicals scavenging ability using the stable radical DPPH at 517 nm. In this study, five different concentrations of 200 μg/mL, 100 μg/mL, 50 μg/mL, 25 μg/mL,12.5 μg/mL, 6.25 μg/mL of each crude extract were prepared by dilution with ethanol. The IC_{50} values watery and ethanol crude extracts of NYOP (L) were 17.14 μg/mL and 15.91 μg/mL.The watery and ethanol crude extracts of NYOP (F) were 35.39 μg/mL and 21.95 μg/mL. These results are shown in Table 3 and Figure 3. Since the lower IC_{50} values, the higher antioxidant activity of the samples occurs. Thus, the ethanol extract of NYOP (L) is greater antioxidant activity than that of NYOP (F) whereas watery extract of NYOP (F) is greater than that of NYOP (L).

Table 3: Radical Scavenging Activity (% RSA) and IC_{50} values of Crude extracts of Leaves and Flowers of NYOP

Test samples	% RSA ± SD at different concentration (μg/mL)						IC_{50} (μg/mL)
	6.25	12.5	25	50	100	200	
NYOP (L) Watery extract	27.36 ± 0.12	41.59 ± 0.81	64.26 ± 0.56	95.34 ± 0.09	101.91 ± 0.28	104.40 ± 0.09	17.14
NYOP (L) Ethanol extract	35.36 ± 0.23	43.36 ± 0.08	67.77 ± 0.05	75.32 ± 0.14	99.36 ± 0.18	112.92 ± 0.18	15.91
NYOP (F) Watery extract	28.36 ± 0.31	36.76 ± 0.61	42.66 ± 0.97	60.31 ± 1.70	70.88 ± 0.61	85.91 ± 0.24	35.39
NYOP (F) Ethanol extract	24.36 ± 0.24	30.68 ± 0.09	56.24 ± 0.19	99.74 ± 0.18	101.45 ± 0.19	103.29 ± 0.37	21.95
Standard Vitamin C	25.20 ± 1.40	53.58 ± 0.88	65.53 ± 1.13	74.82 ± 0.59	83.32 ± 0.78	91.21 ± 0.48	11.70

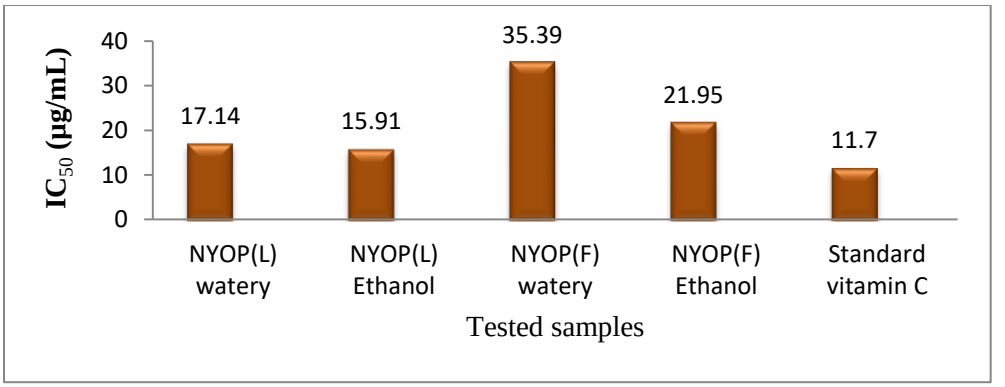

Figure 3: A bar graph of IC_{50} (µg/mL) of watery and ethanol extracts of leaves and flowers of NYOP

Determination of Total Phenolic and Total Flavonoid Contents of Leaves and Flowers of NYOP

The total phenolic contents and total flavonoid contents of NYOP leaves and flowers were estimated by Folin-Ciocalteau method and Aluminium Chloride Colorimetric method respectively. The total phenolic contents in watery and ethanol extracts of NYOP (L) and (F) were found to be 416.2 and 212.2; 320.7 and 195.9 mg of GAE/g of extract respectively. Among these, watery extract of NYOP (L)contained the highest phenolic content. The total flavonoid contents in watery and ethanol extracts of NYOP (L) and NYOP (F) were 88.9 and 114.4; 125.6 and 112.2 mg of QE/g of extract respectively. Among these, watery extract NYOP (F) has the highest flavonoid content. These results are shown in Table 4 and Figure 4.

Table 4: Total Phenolic and Flavonoid Contents of Leaves and Flowers of NYOP

Samples	TPC (mg GAE/g)	TFC (mg QE/g)
NYOP(L) Watery	416.2± 0.02	88.9± 0.01
NYOP(L) Ethanol	212.2± 0.02	114.4± 0.03
NYOP(F) Watery	320.7± 0.01	125.6± 0.01
NYOP(F) Ethanol	195.9± 0.01	112.2± 0.01

TPC = Total phenolic contents TFC = Total flavonoid contents

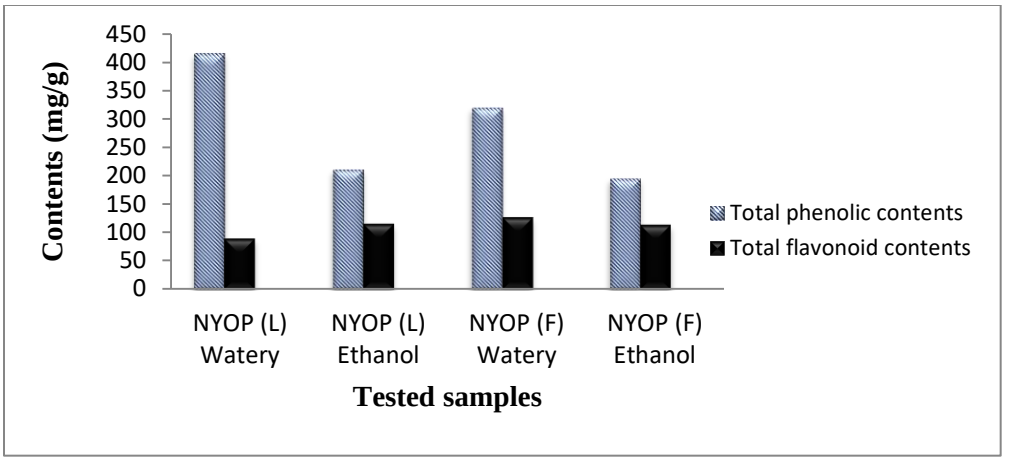

Figure 4: A bar graph of total phenol and flavonoid contents in watery and ethanol extracts of leaves and flowers of NYOP

Conclusion

The following inferences could be deduced from the overall assessment of the chemical investigation on the leaves and flowers of *M. malabathricum*(Nyaung-ye-o-pan).

The preliminary phytochemical tests investigated that alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins, and terpenoids were present in both leaves and flowers of NYOP. The harmful cyanogenic glycosides were absent in both of these samples indicating that these samples are free from toxic effect.

Some nutritional values such as moisture, ash, fiber, fat, protein and carbohydrate contents were observed in both of these samples. The leaves and flowers of NYOP exhibited interesting antimicrobial activities. This information provides a valuable clue for isolation of bioactive compound from leaves and flowers of NYOP.

The results indicated that leaves and flowers of *M. malabathricum* were found to berich in phenolic and flavonoid contents. Total phenolic and flavonoid contents had positive correlation with antioxidant activity. The finding of this study suggested that leaves and flowers of NYOP could be

potential source of natural antioxidant which are great important as therapeutic agents in preventing or slowing the progress of ageing and age associated oxidative stress related degenerative diseases.

Acknowledgements

I would like to acknowledge to Professor Dr. Hnin Hnin Aye (Professor and Head), Professor Dr. Ni Ni Than, Professor, Chemistry Department, University of Yangon, for their kind encouragement and supervisions, and Dr Khin Chaw Win, Lecturer, Chemistry Department, University of Yangon, for close supervisions, invaluable suggestions, helpful advice, patient guidance and encouragement. And I also would like to express profound gratitude to the Department of Higher Education (Lower Myanmar), Ministry of Education, Yangon, Myanmar, for provision of opportunity to do this research and Myanmar Academy of Arts and Science for allowing to present this paper.

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