Study on Phytochemical Constituents and Bioactivities from Bark Extracts of *Bauhinia Malabarica* Roxb. (Chin-Byit)

Myint Myint Khine[1], Kyaw Thu[2] and Khaing Phoo Wai[3]

Abstract

The research focused on the investigation of some phytochemical constituents of *Bauhinia malabarica* Roxb. bark and some of its biological activities. The sample was collected from Hlawkahdar Township, Ayeyawady Region. Preliminary phytochemical tests were conducted with the sample according to test tube methods. The bark sample had relatively the highest content of Ca and K whereas minor components of Ba, Fe, Sr, Mn, Zn, Cu and Br according to EDXRF spectrum. The bark sample was found to contain 13.78 % of moisture, 12.34 % of ash, 3.14 % of protein, 27.37 % of dietary fiber, 0.37% of crude fat, 43 % of carbohydrate and 188 kcal/100g based on dried sample. *In vitro* antioxidant activities of 95% EtOH and watery extracts from *B. malabarica* bark were assessed by DPPH radical scavenging activity assay. The antioxidant activity of ethanol extract (IC_{50} = 1.50µg/ml) was found to be higher than watery extract (IC_{50} = 4.51µg/ml). The *in vitro* antimicrobial activities of PE, EtOAc, $CHCl_3$, 95% EtOH and H_2O extracts from *B. malabarica* bark were screened by agar well diffusion method on six species of microorganisms, namely *Bacillus subtilis, Bacillus pumilus, Candida albicans, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. All extracts except water extracts of bark sample were found to possess antimicrobial activity. From the results of phytochemical constituents, antioxidant and antimicrobial activities of the *B. malabarica* bark observed in the present study, the bark could be applied as the local health remedy for the local indigenous communities of our country.

Keywords: *Bauhinia malabarica* Roxb, phytochemical tests, EDXRF, antioxidant activity, antimicrobial activities

INTRODUCTION

Myanmar has a long history of health care system by herbal and medicinal plants and it has been accepted as a national heritage (San Nyunt Oo, 1993). Herbal medicine is a major remedy in traditional medicine system, which is largely based on the use of roots, leaves, barks, seeds and flowers of the plants.

Botanical aspects of *Bauhinia malabarica*

Family	- Caesalpiniaceae
Myanmar Name	- Chin-byit
English Name	- Mountain Ebony, Orchid-tree, Poor-man's Orchid, Camel's foot
Parts used	- All part of this tree, bark, leaf and flower

Chemical constituents of *Bauhinia malabarica* bark

The chemical constituents of *Bauhinia malabarica* bark vary considerably with variety its, region and age of the product. *Bauhinia malabarica* bark contains several compounds such as tannin, KaempFeral, afzelin, quercetin, isoquercitrin, quercitrin, and hyperoside oil (Salah *et. al.,* 1995).

[1] Associate Professor, Dr, Department of Chemistry, Hinthada University
[2] Lecturer, Dr, Department of Chemistry, Taunggyi University
[3] MSc candidate, Department of Chemistry, Hinthada University

Figure (1). Photograph of *Bauhinia malabarica* Roxb.

Medicinal uses

Leaves, buds and flowers are edible. They are used to treat ailments such as headache, malaria, dysentery and diarrhoeal affections. The bruised bark is applied externally for tumors and wounds such as scrofulous. In India, decoction of the root bark is used as a vermifuge and an infusion of the stem bark as an astringent gargle.

Bauhinia tree parts have anti-bacterial, anti-fungal, anti-malarial, pain reducing, swelling reducing, cytotoxic, fever reducing and thyroid hormone regulating properties. In addition, studies have also shown that the tree is used for treating skin and glandular diseases, leprosy, intestinal worms, tumours, wounds, ulcers, inflammations, scrofula, protoptosis, haemorrhoids, haemoptysis, cough, menorrhagia and bleeding disorders (Mohamed *et. al.*, 2012).

Aim and Objectives of the Present Work

The present research was carried out according to the following objectives.

- to collect and identify the *Bauhinia malabarica* (chin-byit) bark
- to do the preliminary phytochemical test on the collected sample
- to prepare crude extracts from collected sample using polar and non-polar solvents
- to analyze the elements by using EDXRF spectrometry
- to analyze some physiochemical properties of sample such as moisture, ash, fat, carbohydrates, protein and some elements by appropriate analytical methods
- to study the antimicrobial activity of crude extracts by *in vitro* agar well diffusion method and antioxidant activity of alcohol and aqueous extracts using DPPH assay method.

MATERIALS AND METHODS

Sample Collection

The bark sample of *Bauhinia malabarica* Roxb. (chin-byit) was collected from Hlawkahdar Township, Ayeyawady Region in September, 2015. After being collected, the scientific name of the sample was identified by authorized botanists at Botany Department, Hinthada University.

Sample Preparation

The fresh sample was cleaned by washing with water and air-dried. The dried sample was ground using a grinding machine. And then this powdered sample was kept in the sealed air-tight container to prevent moisture changes and other contamination. It was then used without further purification or refining.

Separation of some organic constituents from crude extracts of *Bauhinia malabarica* (Chin-byit) bark

Procedure

Thin layer chromatographic examinations on methanol, ethyl acetate and petroleum ether extracts were performed by using silica gel GF_{254} precoated plate and a variety of solvent systems. TLC plates used in the laboratory were purchased as 20 cm × 20 cm sheets. Each large sheet was cut into plate and it was measured. Using a pencil, a line was drawn across the plate at the 0.5 cm mark. About 1 mg of petroleum or ethyl acetate or methanol extracts was dissolved in 1 ml of a respective solvent. The tip of the capillary tubes was dipped into the solution and then gently touched at the proper location on the TLC plate.

The developing container for TLC was a designed chamber, a beaker with a watch glass on the top. Each solvent (various ratio of petroleum ether or ethyl acetate or MeOH) was poured into the chamber to a depth of just less than 0.5 cm. The beaker was covered with a watch glass, swirled gently, and it was allowed to stand for 5 minutes.

The prepared TLC plate was placed in the developing beaker; the beaker was covered with the watch glass. The solvent was raised up the TLC plate by capillary action. The plate was allowed to develop until the solvent was about half a centimeter below the top of the plate. The plate was removed from the beaker and immediately marked the solvent front with a pencil. The plate was allowed to dry. After developing the chromatograms, these are viewed with the various spraying agents such as Liebermann Burchard reagent, 5 % H_2SO_4 solution, $AlCl_3$ solution, Dragendorff's reagent and 5 % $FeCl_3$ solution to develop colour and classified the fruit constituents.

Some Standardization Parameters for Quality Control of *Bauhinia malabarica* (Chin-byit) Bark

Qualitative elemental analysis of bark sample by EDXRF spectrometry

Energy dispersive X-ray fluorescence spectrometer (Shimadzu EDX-700) can analyze the elements from Na to U. The individual elements comprising in the sample re-emit their own characteristic X-ray. The X-ray detected by using semiconductor detector [Si (Li)] permits multi-element, simultaneous analysis. In this way, the EDX-700 spectrometer qualitatively determines which elements are present in the sample.

About 2.5 g of sample were fabricated into the pellet. The pellet was placed in the sample chamber of EDX-700 spectrometer that can measure the sixteen samples at a time. The chamber was pumped up to vacuum. The pressure was about 38 Pa and the detector temperature is about – 170^{o}C. Therefore, liquid nitrogen needs to be added at the same time of the analysis. Rhodium target was used in EDX-700 spectrometer. Each sample was run for a counting time of about 100 seconds and the spectrum obtained was stored and analyzed in PC based multi-channel analyzer using EDX-700 software. The results were reported and discussed (Table.2).

Nutrient values and physicochemical characterization of bark sample

Procedure

The weighing dishes appearance was checked. Empty dishes and lids were dried in an air oven for 1 hour at 100-105^{o}C. It was cooled in desiccators and weighed to nearest 0.1 mg. Amount of 2-5 g well prepared test portion was placed in dish. It was spread uniformly across the dish, covered and weighed accurately as rapidly as possible to minimize loss of moisture. Lids were removed; dishes and lids were placed in oven as quickly as possible. The sample was dried in an air oven at 130^{o}C for 1 hour. After drying was completed, lids were replaced on dishes and transferred to desiccators and then cooled for 30 minutes. It was weighed accurately until constant weight is achieved. The results were shown in Table (3).

Screening of antioxidant activity of crude extracts from *B. malabarica* bark by DPPH assay

The DPPH (1,1-diphenyl-2picryl-hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of plant materials. This assay has been widely used to evaluate the free radical scavenging effectiveness of various floavonoids and polyphenols in the food system.

In this experiment, the antioxidant activity was studied on 95% ethanol extract, watery extract from selected bark sample by DPPH free radical scavenging assay.

Procedure

The DPPH radical scavenging activity was determined by UV spectrophotometric method. The control solution was prepared by mixing 1.5 ml of 60 μM DPPH solution and 1.5 ml of 95% ethanol using a shaker. The sample solutions were also prepared by mixing thoroughly 1.5 ml of 60 μM DPPH solution and 1.5 ml of test sample solution. The solutions were allowed to stand at room temperature for 30 minutes. After 30 minutes, the absorbance of these solutions was measured at 517 nm by using UV spectrophotometer. The absorbance measurements were done in triplicate for each solution and then mean values so obtained were used to calculate percent inhibition of oxidation by the DPPH method and then IC_{50} (50 % inhibitory concentration) value was also calculated by linear regressive excel program.

Antimicrobial activity screening by agar well diffusion method

The antimicrobial activities of different crude extracts such as pet ether, ethyl acetate, chloroform, 95% ethanol and water extracts were determined against six microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *E. coli* species by employing agar well diffusion method at Central Research and Development Centre, Ministry of Industry, Yangon.

RESULTS

Table (1). Results of Phytochemical Investigation on *B.malabarica* Bark.

Sr.No.	Tests	Extract	Test Reagents	Observation	Remark
1.	α-amino acids	H_2O	Ninhydrin reagent	no pink colour	-
2.	Alkaloids	1% HCl	Mayer's reagent	no white ppt	-
			Dragendorff's reagent	no orange ppt	-
			Wagner's reagent	no brown ppt	-
			Sodium picrate	no yellow ppt	-
3.	Cyanogenic glycosides	H_2O	Sodium picrate solution	no brick red	-
4.	Carbohydrate	H_2O	10% α-naphthol & H_2SO_4	red ring	+
5.	Flavonoids	EtOH	Mg ribbon & conc.HCl	pink colour	+
6.	Glycosides	EtOH	10% lead acetate	white ppt	+
7.	Organic acids	H_2O	Bromocresol green	blue colour	+
8.	Phenolic compounds	EtOH	1% $[K_4Fe(CN)_6]$ & 5% $FeCl_3$	deep blue colour	+
9.	Reducing sugars	H_2O	Benedict's solution	brick-red ppt	+
10.	Starch	H_2O	Iodine solution	no blue colour	-
11.	Saponins	H_2O	Distilled water	no frothing	-
12.	Steroids	PE	Acetic anhydride & conc. H_2SO_4	green colour	+
13.	Tannins	H_2O	2% Gelatin & 1% $FeCl_3$	white ppt	+
14.	Terpenoids	$CHCl_3$	Acetic anhydride & conc. H_2SO_4	no pink colour	-

(+) = presence

(-) = absence

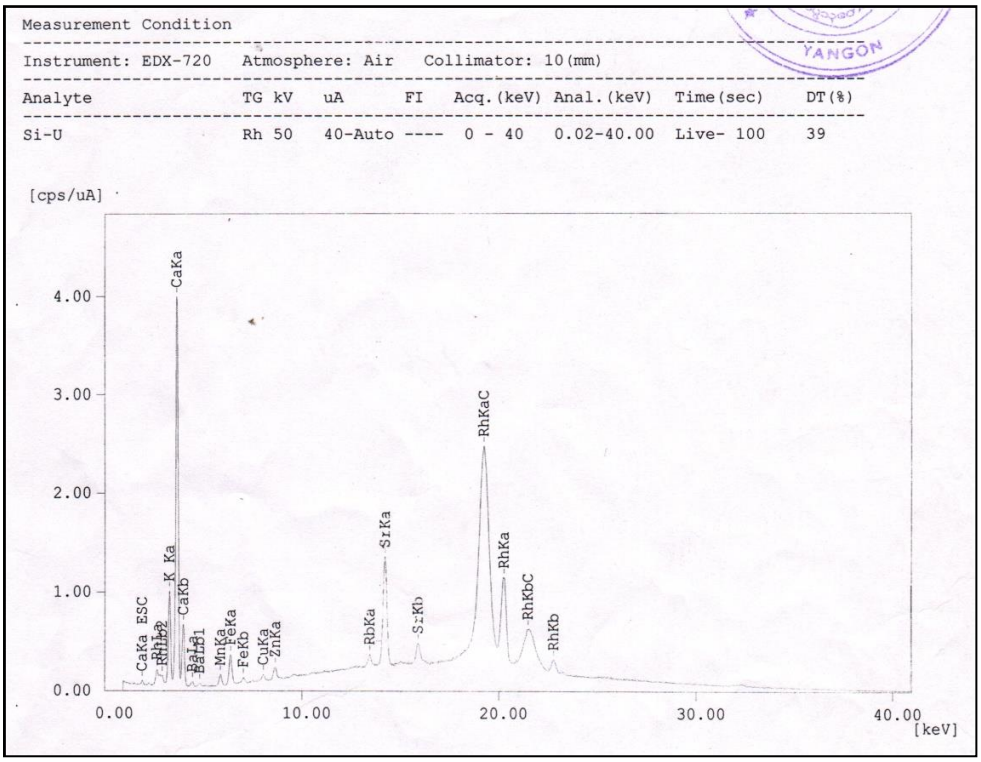

Figure (2). EDXRF spectrum of *B. malabarica* bark.

Table (2). Relative Abundance of Some Elements in *B. malabarica* Bark (By EDXRF).

No	Element	Relative Abundance (%)
1	Ca	1.116
2	K	0.461
3	Ba	0.019
4	Fe	0.012
5	Sr	0.009
6	Mn	0.006
7	Zn	0.002
8	Cu	0.001
9	Rb	0.001

Table (3). Some Nutritional Values of *B. malabarica* (Chin-byit) Bark .

No	Parameters	% Contents (w/w)
1.	Carbohydrate	43.00
2.	Crude Fiber	27.37
3.	Moistures	13.78
4.	Ash	12.34
5.	Crude Protein	3.14
6.	Crude Fat	0.37
7.	Energy Value	188.00 (kcal/ 100g)

Table (4). % Oxidative Inhibition and IC_{50} Values of 95% EtOH and Aqueous Extracts of *B. malabarica* Bark and Standard Ascorbic Acid.

Tested sample	% Inhibition (mean ± SD) in different concentration (µg/ml)						IC_{50} (µg/ml)
	0.625	1.25	2.5	5	10	20	
B. Malabarica EtOH	38.96 ± 0.70	48.13 ± 1.11	57.36 ± 0.31	66.00 ± 0.91	75.44 ± 0.81	80.52 ± 0.02	1.50
B.Malabarica Water	27.90 ± 1.75	37.28 ±1.53	43.91 ± 0.84	51.47 ± 0.71	62.65 ± 0.92	73.56 ±0.93	4.51
Ascorbic acid	25.2 ± 1.40	53.58 ± 0.88	65.53 ± 1.13	74.82 ± 0.59	83.32 ± 0.78	91.21 ± 0.48	1.17

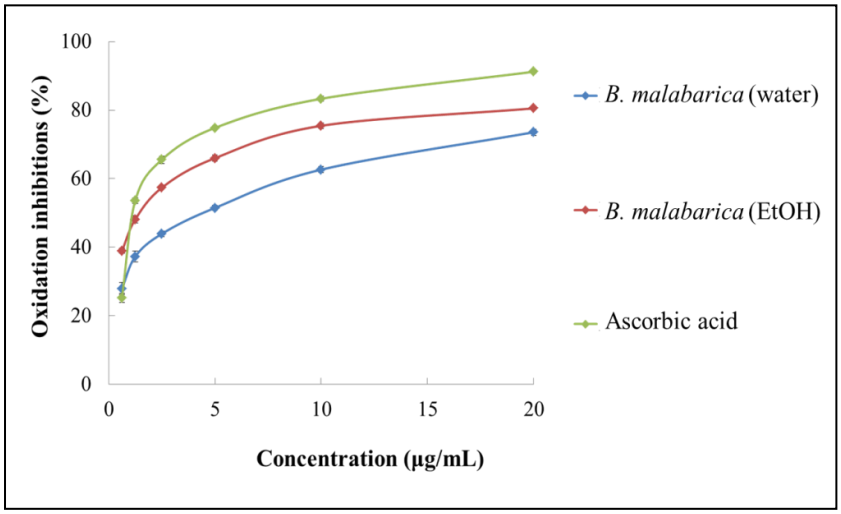

Figure (3). Plot of % oxidative inhibition Vs concentration (µg/ml) of watery and ethanol crude extracts of *B. malabarica* bark in comparison with standard ascorbic acid.

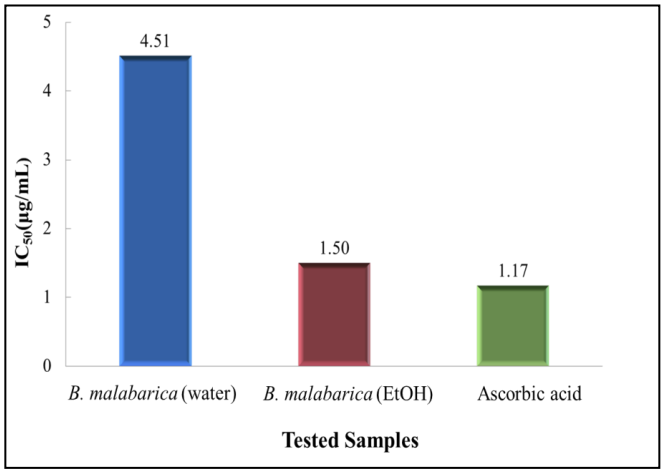

Figure (4). A bar graph of IC50 values of watery and 95% EtOH extracts of B. malabarica bark in comparison with standard ascorbic acid

Table (5). Inhibition Zone Diameters of Crude Extracts of *B. malabarica* Bark against Six Species of Microorganisms

Microorganisms	Types of Microorganisms	Inhibition Zone Diameters (mm)				
		PE	$CHCl_3$	EtOAc	EtOH	H_2O
Bacillus pumilus	Gram (+)ve	-	12	13	-	-
Bacillus subtilis	Gram (+)ve	-	13	14	-	-
Candida albicans	Fungi	-	12	14	13	-
Escherichia coli	Gram (-)ve	-	13	12	-	-
Pseudomonas aeruginosa	Gram (-)ve	-	13	14	-	-
Staphylococcus aureus	Gram (+)ve	-	11	12	-	-

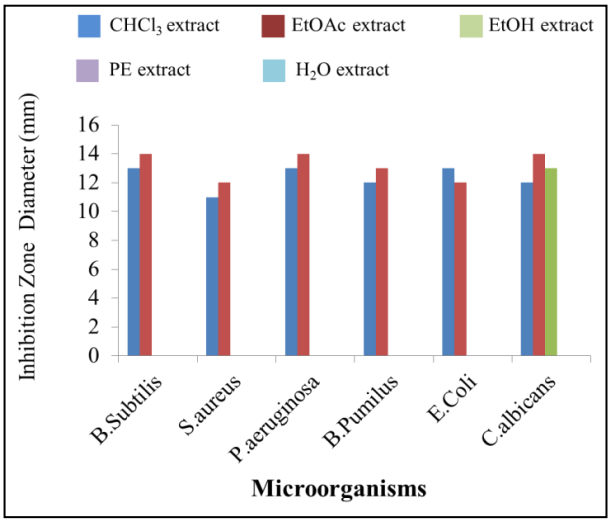

Figure (5). Comparison of inhibition zone diameters for various crude extracts against six microorganisms

CONCLUSION

From the overall assessment of the present work concerning some phytochemical constituents, bioactivities of *B.malabarica* (Chin-byit) bark, the following inferences could be drawn.

(i) The preliminary phytochemical tests on *B.malabarica* (Chin-byit) bark revealed the presence of steroids, flavonoids, glycosides, phenolic compound, carbohydrates, organic acids, reducing sugar and tannin but the absence of α-amino acids, cyanogenic glycosides, starch, saponins, alkaloids and terpenoids in the *B.malabarica* (Chin-byit) bark sample.

(ii) From EDXRF spectrum, Ca, K, Ba, Fe, Sr, Mn, Zn, Cu and Rb were found in the bark of *B.malabarica* (Chin-byit).

(iii) The nutritional values for *B.malabarica* (Chin-byit) bark were found to be 13.78% of moisture, 12.34% of ash, 3.14% of crude protein, 27.37% of crude fiber and 0.37% of crude fat. And 43% of carbohydrate was found to be present in the bark sample and thus *B.malabarica* bark can be utilized as a good source of carbohydrate and fiber.

(iv) According to the antioxidant activity screening of two crude extracts such as ethanol and aqueous extracts from *B. malabarica* bark using DPPH assay, the order of antioxidant activity was as ethanol extracts (IC_{50} = 1.50 µg/ml) > aqueous extract (IC_{50} = 4.58 µg /ml). From these observations, the radical scavenging activity of *B. malabarica* bark ethanol extract was found to be more effective than aqueous extract.

(v) Screening of antimicrobial activity of various crude extracts such as PE, EtOAc, $CHCl_3$, EtOH and H_2O extracts from bark sample was also investigated by employing the agar well diffusion method against *Bacillus subtilis, Staphylococcus aureus, Preudomonas aeruginosa, Bacillus pumilus, Candida albicans and E.coli* species. It was observed that all extracts of *B. malabarica* bark except water extract exhibited inhibition zone diameters between 11-12 mm against *Pseudomonas aeruginosa* species of microorganism tested. $CHCl_3$, EtOAc and EtOH extract of *B.malabarica* bark showed antimicrobial activity against *Bacillus pumilus* and *C.albicans* species ranging the inhibition zone diameter 12-14 mm. Among these, EtOAc extract of *B.malabarica* bark possessed more potent activity, exhibiting the inhibition zone diameters ranging 12-14 mm against all six tested organisms.

Acknowledgements

We would like to express our profound gratitude to Rector, Dr Tin Htwe, and Pro-rector Dr Theigi Shwe, Hinthada University for their encouragement throughout the course of this research work.

We wish to express our sincere thanks to Dr Theingi Nyo (Professor & Head) and Dr Cho Cho Than Professor, Department of Chemistry, Hinthada University, for their kind help with very effective suggestions, advice, comments and encouragement and allowing us to use the facilities in the Department from the beginning till the end of this research work.

References

AOAC, (1990). *Official and Tentative Methods of Analysis, Association of Official Analytical Chemists*. Washington DC: 15th Ed.

Bruice, P. Y. (1995). *Organic Chemistry*. New Jersey: 1st Ed. Prentice Hall Professional Technical Reference

Finar, I. L. (1968). *Organic Experiment*. New York: 4th Ed. D.C Health and Company, 132

Finegold, S. M., W. J. Martin, and E.G. Scort. (1978). *Diagnostic Microbiology*. London: 5th Ed. Mos by Co. Ltd., 586-588

Marini Bettolo, G. B., M. Nicoletti, M. Patamia, C. Galeffic and I. Messana. (1981). "Plant Screening by Chemistry and Chromatographic Procedure Under Field Conditions". *Journal of Chromatography A*, **121**, 213

Mohamed, B. S., S. Ines, S. K. Mohamed, B.Wissem, G. Kamel, C.G. Leila. (2012). "*In vitro* evalution of antioxidant, cytotoxic and apoptotic activities of different extracts from the leaves of Teucrium ramoissimum (laminaceae)", *Journal of Medicinal Plants Research*, **6**(22), 3818-3825

Salah, N., N. J. Miller, G. Pagana, L. Tijburg, G. P. Bolwell and C. Rice-Evans. (1995). "Polyphenolic flavonols as scavenger of aqueous phase radicals and as chain-breaking antioxidants", Arch Biochem Biophy. 2: 339-346

San Nyunt Oo. (1993). "Traditional Medicine Formulary 1000 (Myanmar Version)". Yangon:1st Ed. Ah Thine Ah Wine Press

Trease, G. E. and W. C. Evans. (1980). *Pharmacognosy*. London: 13th Ed. Spottiswoode Ballontyne Ltd.

Vogel, A. I. (1965). *A Text Book of Practical Organic Chemistry*. London: 5th Ed. Language Book Society and Longman Group Ltd.