Screening the Antioxidant and Antimicrobial activities from Bark Extracts of *Bauhinia malabarica* Roxb. (Chin-Byit)

Myint Myint Khine[1], Kyaw Thu[2], 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 detected in the sample according to test tube methods. *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 observed 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 to the local indigenous communities of our country.

Keywords: *Bauhinia malabarica*, phytochemical tests, antioxidantactivity, antimicrobial activities

Introduction

Myanmar is one of the South East Asia countries, has a large number of indigenous medicinal plants. Numerous medicinal plants are reputed to be useful for treatment of various diseases. The study of indigenous medicinal plants and their therapeutics play a very important role in health care system of Myanmar. It 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.

According to the World Health Organization, trading and marketing of medicinal plants and herbal drugs are increasing throughout the world, indicating increasing applications of natural plant materials due to various reasons. Myanmar traditional medicine is widely practiced and well accepted by many Myanmar people, partly as a supplement and partly as an alternative medicine.

Depending on localities and geographic regions, the herbal plants many have different chemical compositions and different efficiency of biological activities. Hence, safety and efficiency data play an important role for the plants, their extracts and active ingredients. Research on medicinal plants has continued to be an area of major interest and priority for researchers in Myanmar for the last several years (Cordell *et.al*.,1989).

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Botanical aspects of *Bauhinia malabarica*

Family	- Caesalpiniaceae
Botanical Name	- *Bauhinia malabarica* ROXB
Myanmar Name	- Chin-byit
English Name	- Mountain Ebony, Orchid-tree
Parts used	- Bark

Tree **bark**

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

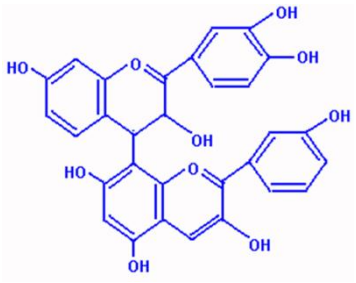
Description and Distribution

Bauhinia malabarica is a small or moderate sized deciduous tree, stocky tree growing to a height of 8 to 10 meters tall. Bark is rough brown, peeling in linear flakes, fibrous, red inside. Leaves are broader than long, 1.5 - 4 inches long, 2-5 inches broad, divided through 1/3 of the length, 7- 9 nerved, slightly heart-shaped at base, rigidly leathery, glaucous and smooth beneath. Flowers are borne in stalkless racemes in leaf axils, 1.5-2 inches long, often 2-3 together, 1/2 inch long, dull-white, often uni-sexual, on very slender stalks, which are l inch long. Pod is 7-12 inches long, 2-2.5cm broad, on a stalk l inch long, flat flexible, many-seed, more or less straight reticulate veins. Seeds are 20-30.

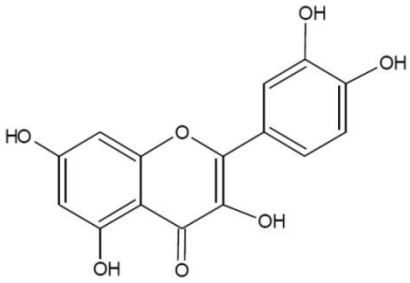
Bauhinia malabarica plants are distributed throughout India, mainly on the sub-Himalayan tracts, Bengal, Assam and in south India. It is found in peninsular India and in the western sub-Himalayan forests, deciduous and semi-evergreen forests, areas receiving 1000 to 3000mm annual rainfall. It also occurs in India to Indo-China, Java and Timor.

Chemical constituents of *Bauhinia malabarica* bark

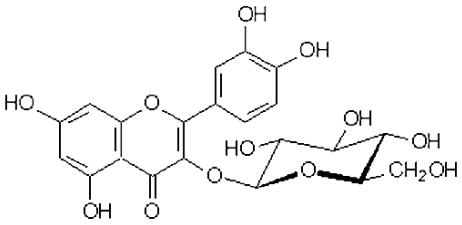
The chemical constituents of *Bauhinia malabarica* bark vary considerably with variety, region and age of the product. *Bauhinia malabarica* bark have been contained several compounds such as tannin, Kaempferol, afzelin, quercetin, isoquercitrin, quercitrin, and hyperoside oil (Salah *et. al.*, 1995).



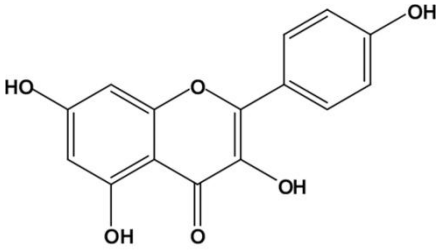
Tannin



Quercetin



Isoquercitrin



Kaempferol

Figure(2) Structures of some chemical constituents of *Bauhinia malabarica* bark

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).

The aim of research was to study antioxidant activity of alcohol and aqueous extracts using DPPH assay method and the antimicrobial activity of crude extracts by*in vitro* agar welldiffusion method.

Materials and Methods

Sample Collection

The bark sample of *B. malabarica*. (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 grounded using 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.

Phytochemical Investigation on the Bark of *Bauhinia malabarica*Roxb. (chin-byit)

Phytochemical investigation on the bark of *B malabarica*. (chin-byit) was screened by test tube method.

Preparation of crude extracts by solvent extraction method

Preparation of aqueous extract

Aqueous extract of bark sample was prepared by boiling 30g of sample with 100ml of distilled water for six hours and filtered. It was repeated three times and the filtrates were combined followed by removal of the water to give watery extract. The extract was dried at normal pressure on a water bath and stored in the refrigerator for the screening of biological activities.

Preparation of 95% EtOH, PE, ETOAc, $CHCl_3$ extracts

For 95 % ethanol, pet-ether, ethylacetate and chloroform extract, the dried powdered sample was percolated with appropriate solvents for one week and filtered. This procedure was repeated for three times. The combined filtrate was concentrated by rotary evaporator to get various extracts. The extract was dried at normal pressure on a water bath and stored in the refrigerator for the screening of biological activities.

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

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 food system (Leea*et. al.*, 2002).

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

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 shaker. The sample solution was 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. 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 equation and then IC_{50}(50 % inhibitory concentration) value were also calculated by linear regressive excel program. (Figure 3 & 4 and Table 2)

$$\% \text{ Inhibition} = \frac{\text{DPPH alone - sample}}{\text{DPPH alone}} \times 100$$

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.

Procedure

The antimicrobial activity of the crude extracts from the bark sample was determined against 6 strains of microorganisms by the agar well diffusion method at CRDC. The extract 1 g was introduced into sterile petridish and dissolved in 1 ml or with least amount of its respective solvent till it was dissolved. The bacteria suspension from tripticase soy broth was done evenly onto the surface of the tripticase soy agar plates immediately after hardening of the agar well were made with a 10 mm sterile cork bore form each seeded agar. After inoculums had been dried for 5 minutes, the agar disc was removed and the wells were filled with sample to be tested. And then, the plates were incubated at 37°C. After overnight incubation at 37°C, the diameter of inhibition zone including 10 mm wells was measured. The well plate dilution method was used to test antimicrobial action of the extracts on 24 hours broth culture of the organisms used. The extracts from the sample were tested with six microorganisms. These results are reported in (Figures5 and Table 3).

Results and Discussion

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

Screening of Antioxidant Activity of Crude Extracts from *B. malabarica* (Chin-byit) Bark

In this experiment, six different concentrations (0.625 µg /mL, 1.25 µg /mL, 2.5 µg /mL, 5 µg /mL, 10µg/mL and 20 µg /mL) of each crude extract were prepared in ethanol solvent. Ascorbic acid was used as standard and ethanol without crude extract was employed as control.

Table 2 % 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

From these results, it can be clearly seen that IC_{50}values were found to be 1.50µg/mL for ethanol extract, and 4.51 µg/mL for aqueous extract. Among these extracts, since the lower the IC_{50} showed the higher the free radical scavenging activity. Ethanol extracts were found to be more effective than aqueous extracts in free radical scavenging activity. However, it was observed that all of these extracts have the lower antioxidant activity than standard ascorbic acid (IC_{50} -1.17 µg/mL).

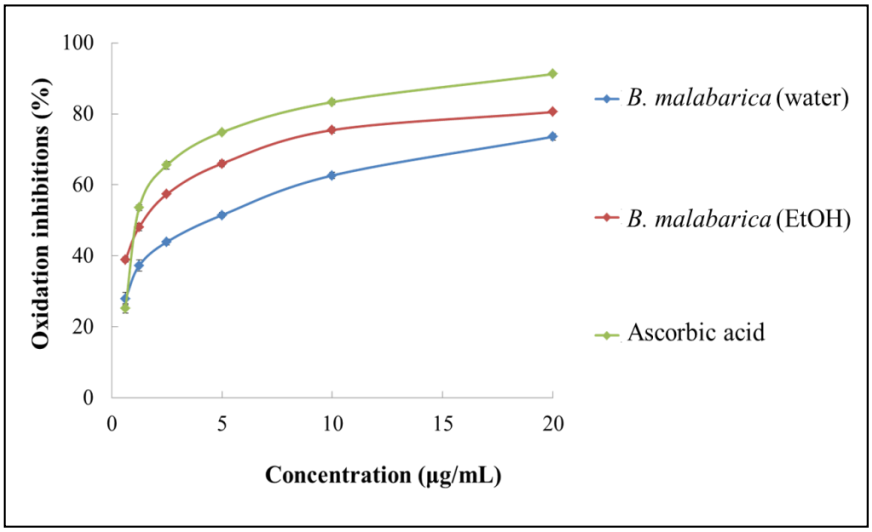


Figure 3 Plot of % oxidative inhibition Vs concentration (µg/mL) ofwatery and ethanol crude extracts of *B. malabarica*bark incomparison with standard ascorbic acid

Antimicrobial Activity of *B. malabarica* Bark

Screening of antimicrobial activity of crude extracts such as petroleum ether, ethyl acetate, chloroform, ethanol and water extracts from *B.malabarica* bark samples were done by agar well diffusion method. . The measurable inhibition zone diameter of crude extracts showed the degree of antimicrobial activity (Figures 5&6 and Table 3).

Table 3 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	-	-

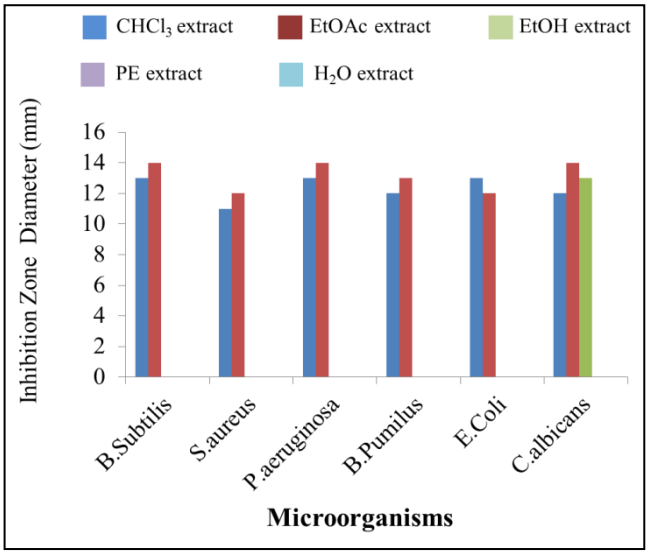


Figure 4 Comparison of inhibition zone diameters for various crude extracts against six microorganisms

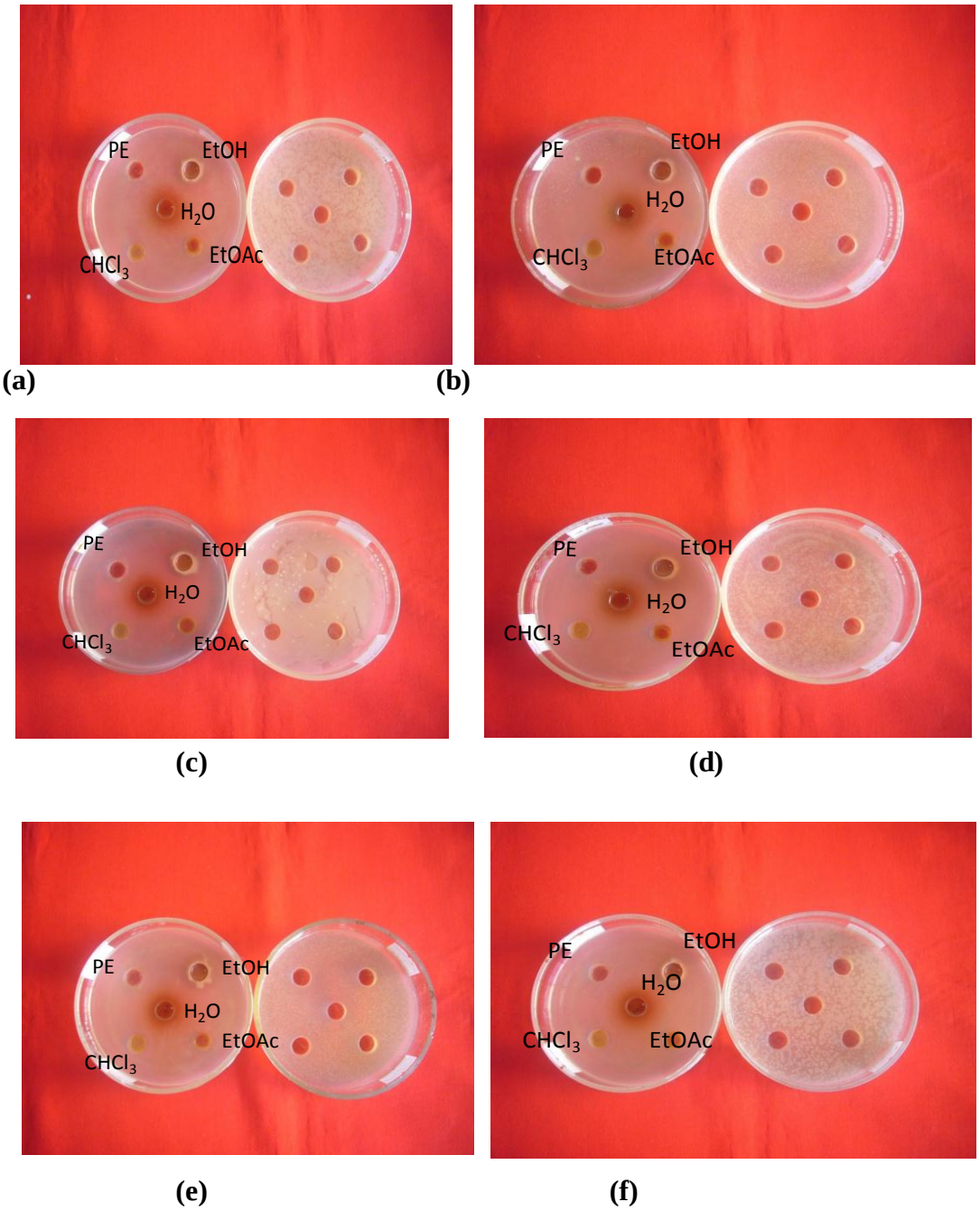


Figure 6 **Inhibition zones of various crude extracts against**
(a) *Bacillus subtilis* (b) *Staphylococcus aureus*
(c) *Pseudomonas aeruginosa* (d) *Bacillus pumilus*
(e) *Candida albicans* (f) *Escherichia coli*

From the results 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 diameter12-14 mm. EtOAc extracts of *B.malabarica* bark showed inhibition zone diameters ranging 11-14 mm against all tested organisms. Thus it was found to be more potent than the other extracts. All extracts except water extract exhibited

inhibition zone diameters between 11-14 mm against microorganism tested. Therefore these extracts may have broad spectrum activity. The inhibition zone diameter 14 mm of EtOAc extract showed the highest activity against *Bacillus subtilis* and *Staphylococcus aureus.* Thus it can be effectively used as active remedy for this treatment of their related diseases.

Conclusion

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

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

(ii) 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.

(iii) Screening of antimicrobial activity of various crude extracts such as PE, EtOAc, $CHCl_3$, EtOH and H_2O extracts from bark sample was also investigation by employing 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 b*ark 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, exhibition the inhibition zone diameters ranging 12-14 mm against all six tested organisms.

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