Original Article

Antioxidant, Antifungal, And Phytochemical Analysis of *Bauhinia Malabarica:* An In-vitro Study

Mahendra Sharma[1], G. Neerajarani[2], Mujeeb C. A[3], Anu V[4], B. Sravan[5], Arunoday Kumar[6]

[1,2]Senior lecturer, [3,4,5]Postgraduate Students, [6]Senior Lecturer

[1]Department of Biochemistry, [6]Department of Prosthodontics and crown & bridge, Hazaribag college of dental sciences and hospital, Demotand, Jharkhand, India. [2]Department of pharmacology, Sree Sai dental College, Srikakulam, Andhra Pradesh, India. [3,4,5]Department of Biochemistry, Mangalore University, Mangalore, Karnataka, India.

Corresponding Author: Mahendra Sharma, MSC (Biochemistry), Department of Biochemistry Hazaribagh Dental College, India. **E-mail:** mahendra152207@gmail.com

Abstract

Introduction: Infectious diseases are still a major threat to public health, despite the tremendous progress made in human medicine. The medicinal properties of several herbal plants have been documented in ancient Indian literature and the preparations have been used effectively in treatment of several diseases. The present study reports the phytochemical, antimicrobial and antioxidant activities of Bauhinia Malabarica.

Material & Methods: Leaf extracts and washed with tap water and for the removal of soil and dust particle then the extracts were dried in the oven at 400c to get thick paste. Then the collected samples were stored at 40C f the obtained extract was divided into two parts. One part was kept at 80c for overnight and another one part was kept at room temperature. After that various test are applied for phytochemical analysis like saponins (Foam Test), Test for phenolic compounds many more. Antioxidant activity: DPPH▪ stable free radical scavenging assay, free radical scavenging activity by ABTS method are used. Antimicrobial activity: The analysis of minimum inhibitory concentration against fungal species methods are used.

Result: In phytochemical analysis studies, the extracts prepared with hexane, chloroform, methanol, PBS RT and PBS 80C showing presence of many kinds of phytochemicals saponins. Antioxidant activity: five extracts, Methanol, chloroform, Hexane, PBS RT, PBS 80C are showing the free radical scavenging activity. In Antimicrobial study against salmonella, E. coli and P. aerogenosa did not showed any clear zone of inhibition for any of the extracts except standard marker.

Conclusion: *B. Malabarica* has many pharmaceutical compound which could be helpful to design certain drugs.

Keyword: Antimicrobial, Antioxidant, Bauhinia, Medicinal plants

Introduction:

Medicinal plants are a science of life and natural products. Medicinal plants are of great importance to the health of individuals and communities, many people in the world have difficulty in gaining access to modern medicines; they use traditional medicines, based on the use of medicinal herbs and plants as an alternative to a conventional treatment for their recovery.[1,2] The medicinal properties of several herbal plants have been documented in ancient Indian literature and the preparations have been used effectively in treatment of many diseases.[3] *Bauhinia malabarica* is a small or moderate sized deciduous tree. *Bark* is rough brown, peeling in linear flakes, fibrous, red inside. Leaves are 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.[4] Flowers are borne in stalk less racemes in leaf axils, 1.5-2 inches long. Flowers are 1/2 inch long, dull-white, often uni-sexual, on very slender stalks. Male and female flowers are usually on different stems. Sepal cup has

five equal triangular teeth. Petals are spade-shaped, equal. Pod is 7-12 inches long, 2-2.5 cm broad, on a stalk 1 inches long, flat flexible, many-seeded, more or less straight reticulate veins, which starting diagonally from both sutures meet in the middle.[5,6] *B malabarica* has many medicinal properties;[7] Leaves are used as flavouring for meat and fish and applied to the forehead for fevers. Good source of calcium and iron. Decoction of this plant root bark used for liver problems. Root and stem of this plant used for the treatment of cholera would heal diuretic and dysentery.[8] No work has done even those study for various medicinal properties so not report regarding the phytochemical analysis, antimicrobial activity and antioxidant activity. Leaves has taken for the present study and aimed that scientific validation of traditional uses.[9] The present study was carried out to study the study reports the phytochemical, antimicrobial and antioxidant activities of Bauhinia malabarica.

About the plant:[5,6,7]

- ✓ **Kingdom:** Plantae,
- ✓ **Class:** Angiosperm,
- ✓ **Order:** Fabales,
- ✓ **Family:** Fabaceae,
- ✓ **Subfamily:** Caesalpiniaceae,
- ✓ **Genus:** Bauhinia,
- ✓ **Species:** malabarica

Plant name in different languages:

- ✓ **English:** Malabar mountain ebony,
- ✓ **Hindi:** Almosa,
- ✓ **Kannada:** Basavanapada

Materials and Methods:

Chemical used for the reagents and media were produced from Hi-media laboratory Pvt ltd, and Mark India limited, Mumbai.

Glassware:

- ➢ Conical flask
- ➢ Petri plates
- ➢ Test tube
- ➢ Micro pipette and tips

Chemicals used:

Hexane, chloroform, methanol, NaoH, sulphuric acid, Hydrochloric acid, acetic acid, ferric chloride, iodine, Benedicts reagent, potassium hydroxide, formic acid, silica gel, DPPH(2, 2-Diphenyl-1picrylhydrazil), DNS, folin-ciocalteu, sodium carbonate, ascorbic acid, DMSO, ethanol, Disodium hydrogen phosphate, methylene blue, Hydrogen peroxide, tannic acid, ascorbic acid, H_2O_2, ammonium molybdate, disodium phosphate, sodium di hydrogen, potassium ferricyanide, TCA, ABTS, etc.

Instrument used:

Soxhlet apparatus, Spectrophotometer, LAF, incubator, TLC set, cooling centrifuge, autoclave, Hot air oven.

Media Used and their Composition:

Table no. 1, 2 & 3 shows the media and their composition.

Sample collection:

The selection of plant materials and sampling area were kusalnagar, the plant *B. Malabarica* is traditionally well known for its medicinal property. But the much of work has been not reported for this plant. The leaves of *B. malabarica* were collected from the kusalnagar during the month of February 2012.

Preparation of solvent extracts:

The fresh leaves were washed with tap water and for the removal of soil and dust particle. Then washed with 0.1 sodium hypochlorite, this was followed by deionised water 2-3 times. Then leaves were shade dried for a week then powdered.100g of this powder used for solvent extraction by soxhlet apparatus using solvents (hexane, chloroform, methanol). The extracts were dried in the oven at 400c to get thick paste. . Then the collected samples were stored at 4^0C.[10]

PBS extracts:

The extract prepare by using phosphate buffer saline. Fresh leaves were washed with sodium hypochlorite and water and ground with PBS buffer (W/V:1:6). The obtained extract was divided into two parts. One part was kept at 8^0c for overnight and another one part

was kept at room temperature. After incubation, solutions were filtered through double layer muslin cloth, filtrates were collected and centrifuged at 5000rpm for five minutes and kept it in evaporation. The prepared solvent extracts and the PBS extracts were stored in the 4^0c for further use.

Phytochemical Analysis:

Qualitative tests for phytochemical analysis Test for saponins (Foam Test):[11]
2ml of the extracts diluted to 20ml of the distilled water and agitated in graduated cylinder for 15 minutes. The formation of the foam layer indicates the presence of saponins.

Test for lipids (saponification test):[12]
2ml sample was boiled with 3ml of alcoholic KOH and mixed using vertex. The tube was kept in boiling water bath for 15-20 minutes. The formation of foam was indicated the presence of lipids.

Test for tannins (ferric chloride test):[13]
0.5ml of the sample was taken and dissolved in 20ml of the water. To this 0.1% ferric chloride was added, the presence of brownish green or blue black colour indicates the presence of tannic.

Test for Terpenoids (Salkowski test):[14]
5ml of extract mixed with 2ml of chloroform in test tube. 3ml of Concentrated Sulphuric acid was added to the sides of the tubes. The presence of the red-brown colour at the inter face indicates presence of the Terpenoids.

Test for protein (Ammonium Sulphate saturation test):[14]
Few amount of the sample was taken and saturated with Ammonia per Sulphate. The white precipitate indicates presence of Protein.

Antioxidant Activity:

DPPH Stable Free Radical Scavenging Assay:[15]
The DPPH radical scavenging activity of *B. malbarica* extracts and ascorbic acid (Standard) were determined with slight modification. The stock solution was prepared by using 0.1mM DPPH in ethanol and standardize to 1.9 OD at 517nm. The standard (ascorbic acid) and sample was taken separately in different aliquots (60µl) and make up to 1ml using ethanol. The concentration of the extract used was (0-500µl/ml). To this 2ml of the .1mM DPPH was added and incubated in dark for 30 minutes at room temperature. Absorbance was read at 517nm and percentage of the inhibition was calculated using formula.
Radical scavenging Assay = ADPPH-A sample/ADPPHX100

ADPPH is the absorbance of DPPH radical + ethanol and a sample is the absorbance DPPH radical + sample or standard. The IC50 (concentration providing 50% inhibition) value of the standard and extract was determined using regression curves in the linear range of concentrations. The antioxidant activity index (AAI) was calculated using formula.
AAI= {DPPH}µg/ml/IC50 µg/ml

Free Radical Scavenging Activity By ABTS Method:[16]
Determination of antioxidant activity by ABTS assay, the procedure with some modification. The stock solutions included 7.5mM $ABTS^{\cdot+}$ solution and 3mM potassium persulfate. The working solution was prepared by mixing equal volume of two stock solutions and allowing them to react for 12-16hrs at room temperature in dark. The stock solution was then diluted by mixing 1ml $ABTS^{\cdot+}$ solution with 60ml of methanol to obtain an absorbance of 1.1±0.02 at 734nm. Fresh $ABTS^{\cdot+}$ was preferred for each assay. 0-10µg/ml ascorbic acid and 0-30µg/ml plant extracts were taken for assay. 1ml of freshly prepared $ABTS^{\cdot+}$ was added and incubated in dark for 2h. Then the absorbance was read at 734nm using a spectrophotometer. The % of inhibition was calculated.

Anti-Microbial Activity:
Salmonella, *Escherichia coli and P.* aerogenosa were used for antimicrobial and fungal strain Aspergillus nigar and penicillum sp are used for the antifungal

activity. All pathogens were tested for purity and used. For antimicrobial evaluation, agar cup diffusion method, Nutrient agar plates were used for the antibacterial study and for fungal potato dextrose agar media used. The plates were evenly spread by using cotton swab and agar cups were prepared by scooping out the media with cork borer. The wells were filled with 10-30µl of crude extract dissolved in DMSO (6mg/ml). Standard tetracycline (1mg/1ml) and candida 200µl/1ml used for bacteria and fungi respectively .Plates were incubated at $37^{O}C$ for bacteria and for fungi at room temperature and zone of inhibition were measured and recorded.

The Analysis of Minimum Inhibitory Concentration against Fungal Species:

MIC is the lowest concentration of an antifungal that will inhibit the visible growth of a microorganism after overnight incubation. Determination of MIC was done according to the method of (Xiaoxi,2011).[17] The 10 dried test tubes taken and 1ml of potato dextrose broth was added to all the test tubes.50µl of inoculums was added to all the test tubes. Then 2ml of plant extract was added to the first test tubes. 1ml of sample was taken from the 1st test tube which is serially diluted with next tube. After completion of transferring the tubes were shaken properly and placed in incubator at 37°c for 18-24 hrs. After the incubation the minimum concentration was observed and reading was taken at 620nm.

Results:

Phytochemical analysis:

In phytochemical analysis studies, the extracts prepared with hexane, chloroform, methanol, PBS RT and PBS $8^{0}C$ showing presence of many kinds of phytochemicals saponins, phenolic compound, flavonoids, terpinoids, tannins, glycosides, carbohydrates and proteins. Phenolic compound and carbohydrates were present in all extracts except hexane. Steroids and lipids were not found in any types of the leaf extracts as given below **(Table No. 1)**.

Qualitative Analysis of phytochemicals

Determination of total phenolic content:

The total phenolic or tannic content of different extracts of the *B. malabarica* was determined by the method of [6] with slight modifications. The total phenolic content of methanolic and PBS $8^{0}C$ extracts were estimated by the Folinciocalteau method. Total phenol found to be maximum in methanolic extracts was little higher than chloroform extract. The methanolic extract **(Graph No. 2)** shows little higher % of this phytochemicals with total phenolics of 24µg of standard **(Graph No. 2)**.

Antioxidant Activity

Free radical Scavenging Assay

Reactive oxygen species (ROS) is a term which encompasses all highly reactive, oxygen-containing molecules, including free radicals. Main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources.

The DPPH radical scavenging activity of B. malbarica extracts and ascorbic acid (Standard) were determined according to the method 15. Out of five extracts, Methanol, chloroform, Hexane, PBS RT, PBS 80C are showing the free radical scavenging activity. Methanol and chloroform extract showing more activity. Among all methanol extract had high activity. The concentration necessary for 50%reduction of free radicals the methanol extract showed better radical scavenging activity than the other extracts as shown in **Graph No 4 and 5**. The IC50 value by DPPH method was as follows Standard ascorbic acid was 3µg, Methanolic extract was 18µg, and by ABTS it was found to be as follows Standard ascorbic acid was 16µg, Methanol extract was 24µg as given below **(Table No. 2)**.

Free radical scavenging activity by ABTS method

In ABTS method, Methanol and chloroform extract was given more activity compared to the other extracts. The PBS RT and PBS 20 shows the less antioxidant activity compared to methanol and chloroform extracts as given in the **Graph No. 6**

(standard, ascorbic acid) and **Graph No. 7** showed the high antioxidant activity of methanol followed by chloroform extract.

Total reducing power:

The total reducing power of the plant extracts of B. malabarica was measured according to the method of 14, by potassium ferric cyanide assay. The total reducing power in six plants extracts. The methanol extract had higher reducing power and followed by PBS RT and chloroform **(Graph No. 8)**.

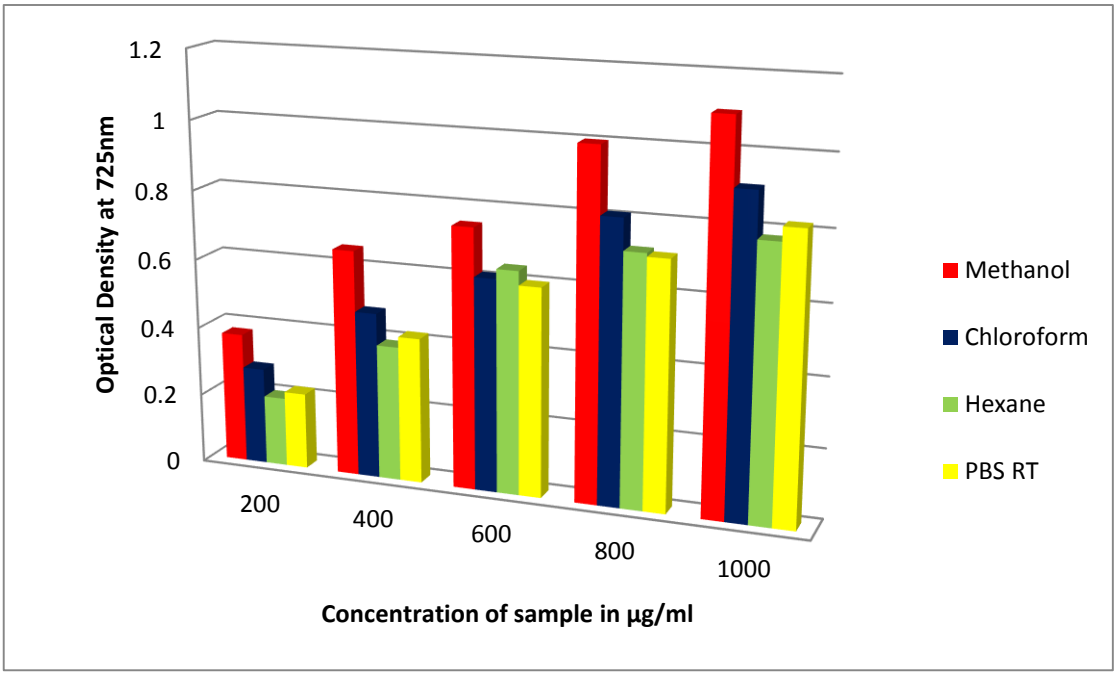
Antifungal activity of plant extracts:

Leaf extracts of B. malabarica showed active result against antifungal properties as showed in **(Figure No. 1, 2, 3, and 4)**, **(Graph No. 9 & 10)** on methanol and PBS RT extracts. The total five plant extracts were taken for antifungal activity against Aspergillus niger, penicillin. However pythium spices has less sensitive compared to a. niger sps and penicillin spices.

Table No 01: Phytochemical Analysis of *B. Malabarica* Extracts

Name of the tests	Hexane	Chloroform	Methanol	PBS-RT	PBS 8^0C
Saponins	-	+	-	+	+
Phenolic compound	-	+	++	+	+
Flavonoids	-	-	-	+	+
Glycosides	-	+	-		-
Carbohydrates	-	+	++	+	+
Lipids	-	-	-	-	-
Tannins	-	-	+	+	+
Steroids	-	-	-	-	-
Terpenoids	+	+	+	+	+
Proteins	-	-	-	+	+

Graph No. 1: Total Phenolic Content of the Plant Extracts



Graph No. 2: Phenolic Content of Tannic Acid (Standard)

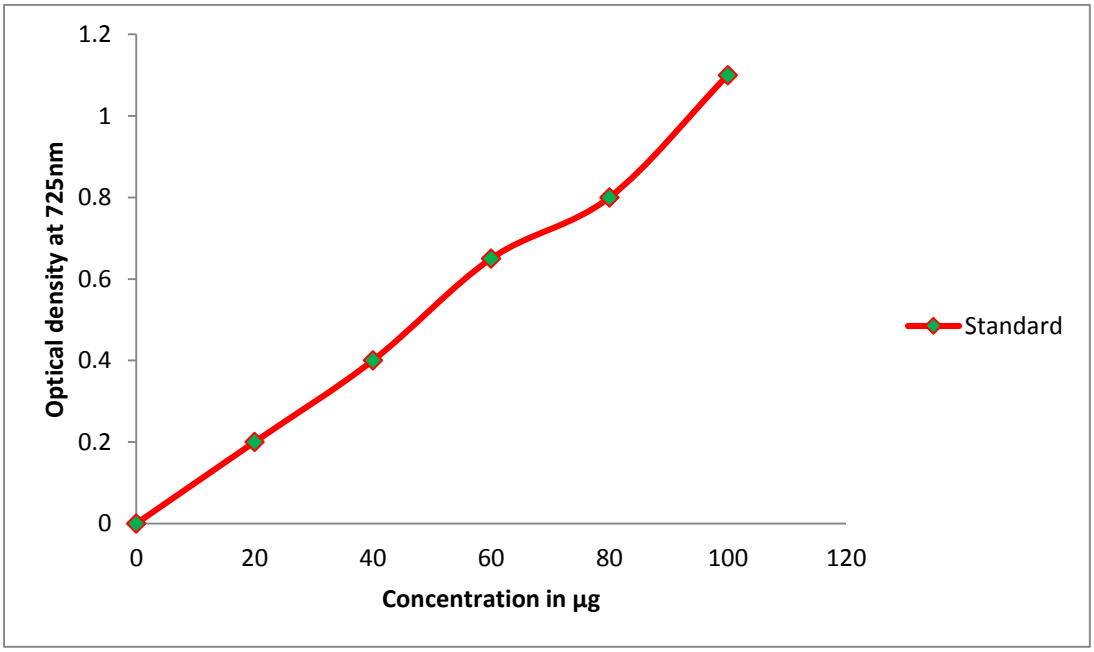
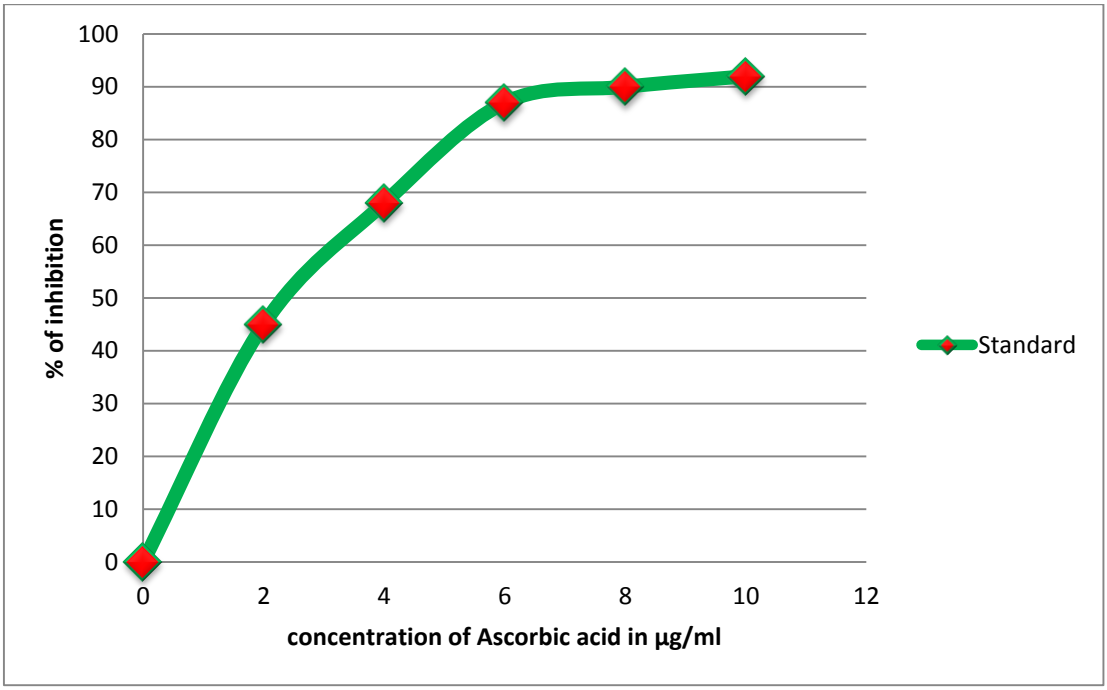


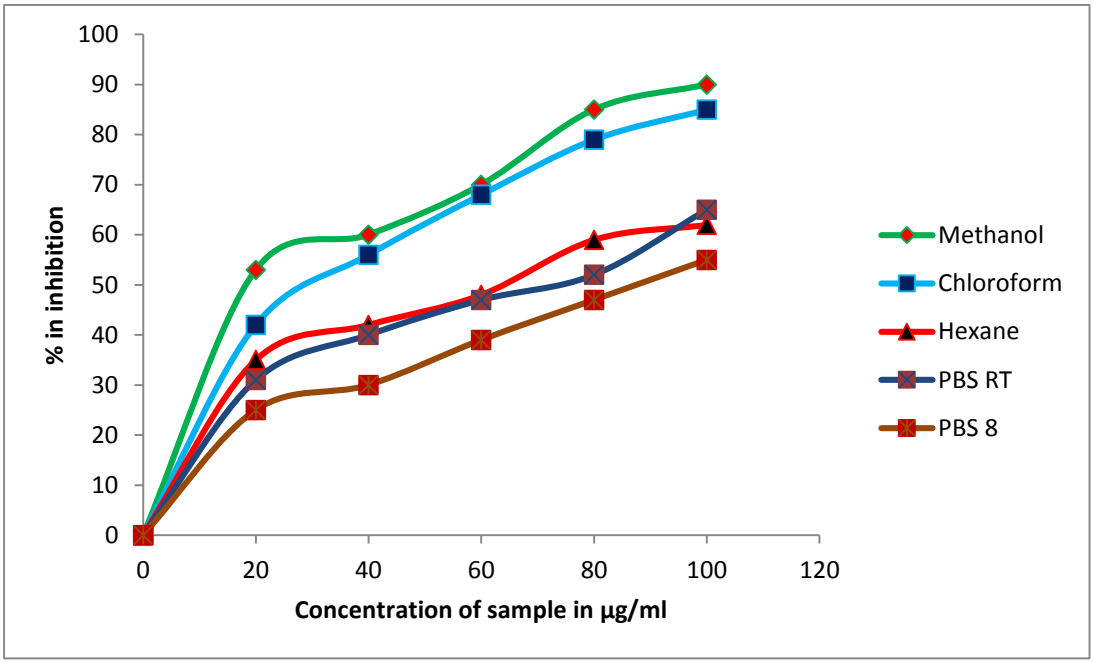
Table No. 2: IC Value of the Antioxidant Scavenging Assay

Extracts	IC_{50} value
Standard	3µg
Methanol	18µg
Chloroform	24µg
Hexane	53µg
PBS RT	55µg
PBS 8^0C	57µg

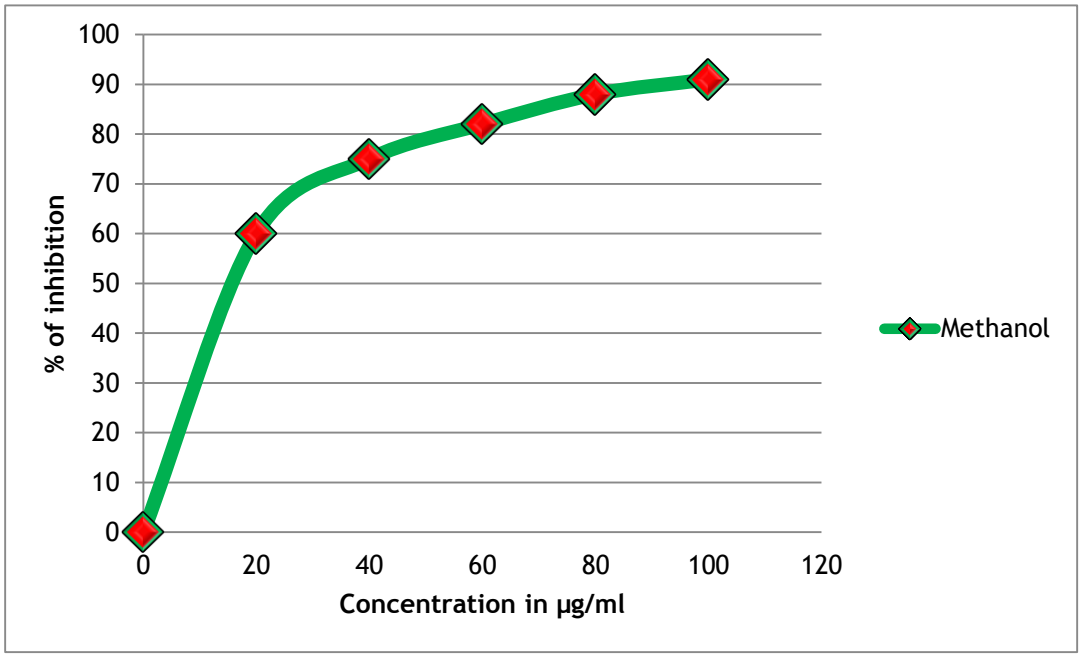
Graph No. 4: Percentage Inhibition of Free Radical Scavenging Assay by DPPH Method by Standard Ascorbic Acid



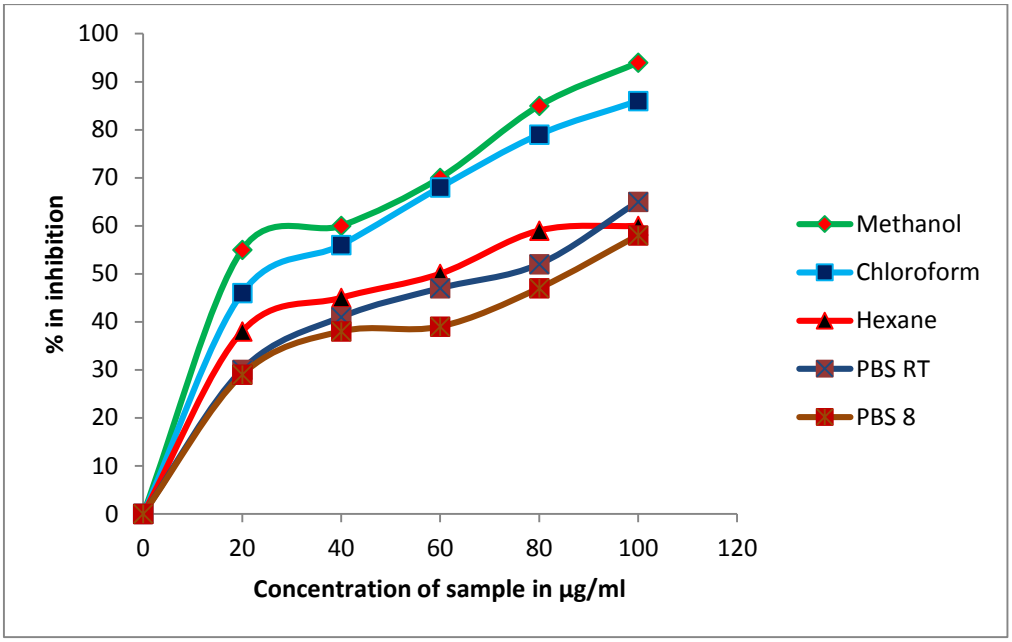
Graph No. 5: Percentage of Inhibition of Free Radical Scavenging Assay by DPPH Method



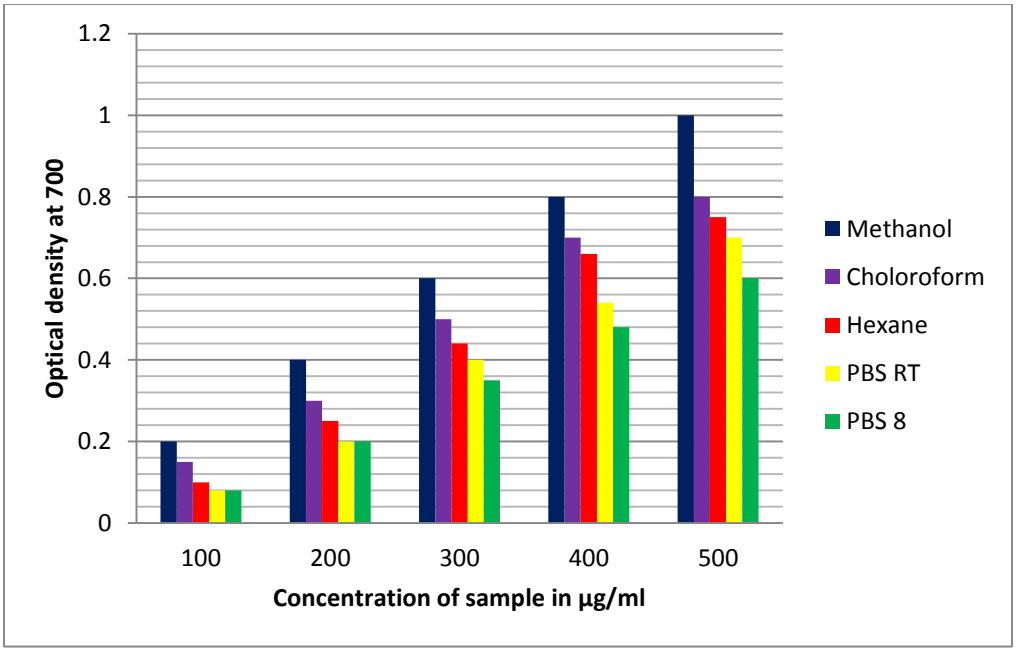
Graph No. 6: Percentage of Inhibition of Free Radical Scavenging Assay by ABTS Method by Standard Ascorbic Acid



Graph No. 7: Percentage of Inhibition of Free Radical Scavenging Assay by ABTS Method



Graph No. 8: Total Reducing Power of the Plant Extracts



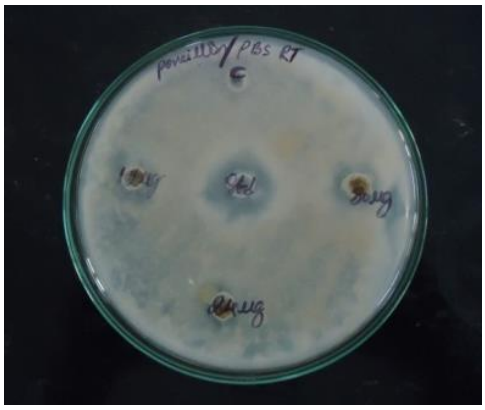


Figure No. 1: PBSRT-Penicillin sp

Figure No. 2: Methanol-penicillin sp

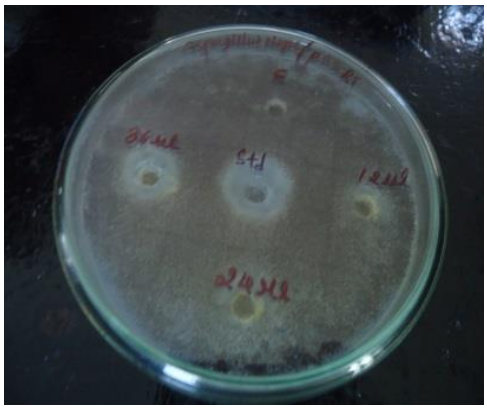


Figure No. 3: PBS RT-A .Niger sp

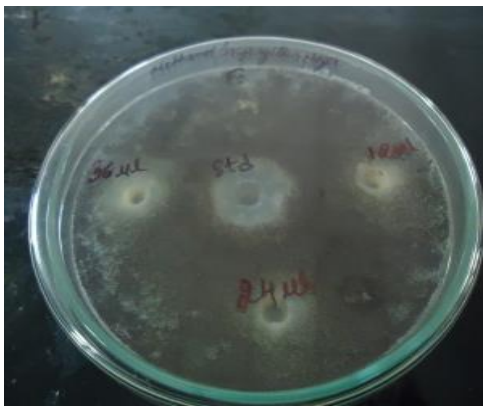
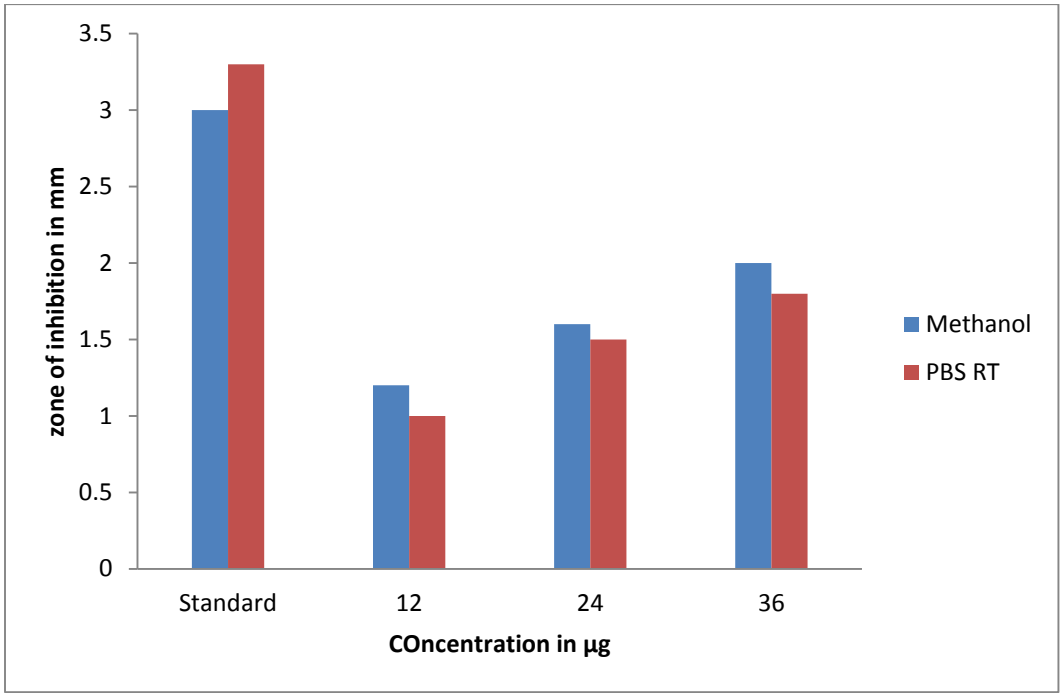


Figure No. 4: Methanol-A Niger sp

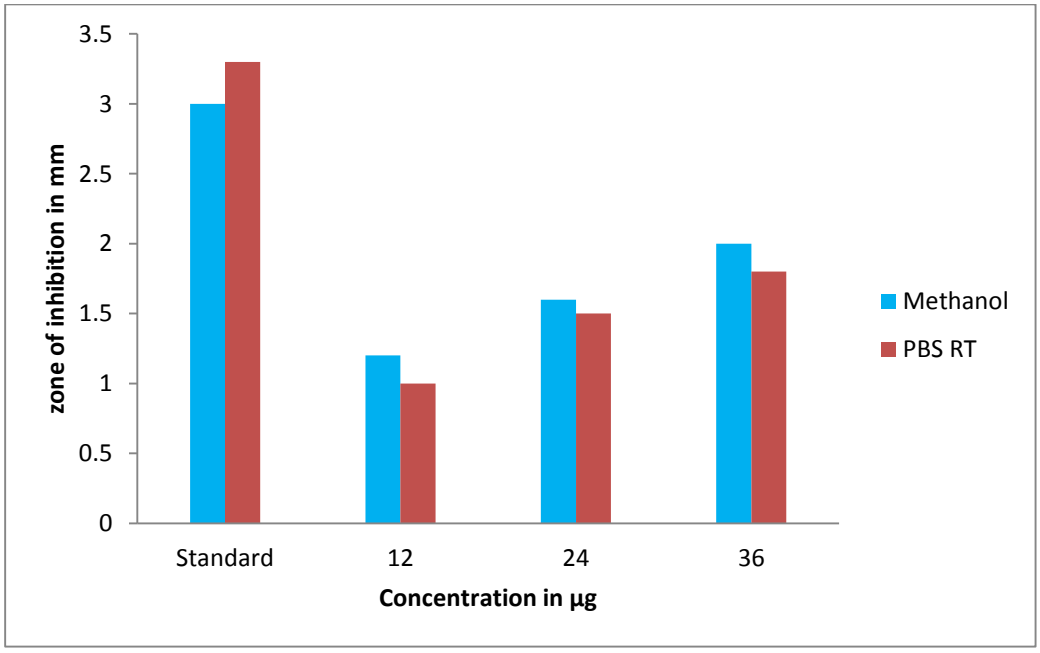
Table No. 3: Antifungal Activity of the Methanol and PBS RT against Fungal Species

Extract Name	Aspergillus niger sps(mm)				Penicillin sps(mm)			
Methanol	**Std**	**12µg**	**24µg**	**36µg**	**Std**	**12µg**	**24µg**	**36µg**
	3	1.8	2.0	2.4	3	1.2	1.6	2.0
PBS RT	3.3	0.8	1.0	1.4	3.3	1.0	1.5	1.8

Graph No. 9: Antifungal Activity of Methanol and PBS RT Extract against Aspergillus niger



Graph No. 10: Antifungal Activity of Methanol and PBS RT Extract against Pencillium Sps



Discussion:

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs .A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties.[19] In present study five different extracts showed presence of various kinds of phytochemical, terpinoids, phenolic compounds, flavonoids, protein, carbohydrates and flavonoids found in almost all the extracts except hexane. The total phenolic content was high in the methanolic extract. After the phytochemical analysis the plant extract was studied for Antioxidant activity. The radical scavenging activity was carried out by two sensitive methods DPPH and ABTS. The Antioxidant activity of different extracts was studied. Methanol and chloroform extracts showed good Antioxidant property. Methanol extracts showed the highest total phenolic content and reducing power compared to other extracts. Antifungal activity of this plant extracts indicated that the plant have biomolecules to combat with pathogenic fungi in antifungal study. In oral environment it would be beneficial to combat with the fungal diseases like oral candidiasis etc

Conclusion:

Plants are major source for pharmaceutical chemical to design new drugs for many diseases. B. malabarica has many pharmaceutical compound which could be help to design certain drugs. However more experiments needed to isolate and characterization of compounds.

References:

1. Ames BN, Shigenaga,M.K. and Hagen, T.M. Oxidants, Antioxidants and Degenerative Diaeases Of Aging. Nat. Aca. Sci. 1988;49:,915-2.
2. Aiyegoro AO, Okoh AI. Preliminary Phytochemical Screening and In Vitro Antioxidant Activities of The Aqueous Extract Of Helichrysum Longifolium. Nat. Aca. Sci. 2010;56:,305-0.
3. Agnihotri, JP. In Ancient and Medieval History of Indian Agriculture. 2000;41:481-9.
4. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity occurrence, and potential uses. Food Chem 2006;99(1): 191-203.
5. Barnes J, Anderson L, Phillipson J. St John´s wort (Hypericum perforatum L.): a review of its chemistry, pharmacology and clinical properties. J. Pharm. Pharmacol.2001;53(5): 583-600.
6. Bocco A, Cuvelier M, Richard H, Berset C. Antioxidant activity and phenolic composition of citrus peel and seed extracts. J. Agric.Food Chem. 1998; 46(6): 2123-9.
7. Brauggler J, Duncan CA, Chase LR. The Involvement of Iron in Lipid Peroxidation Importance of Ferrous to Ferric Ratio in Initiation. J of Bio. 1986; 261: 101-2.
8. Chandra AM, Ibrahim D, Sulaiman SF. Antioxidant, Antimicrobial activity and Toxicity Test Of Pileamicrophylla Plant Cell. 2010;78:137-3.
9. Chopra RN, Nayer SL, Chopra IC. Medicinal Plants In Glossary Of Indian Medicinal Plants. 1956; 4: 157- 160.
10. Parekh J, Chanda SV. In vitro Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. Turk J Biol, 2007; 31: 53-58.
11. Mallikharjuna PB, Rajanna N, Seetharam YN, Sharanabasappa GK. Phytochemical Studies of Strychnos potatorum L.f.- A Medicinal Plant: E-Journal of Chemistry, 2007; 4(4): 510-8.
12. Dimberu G, Bedemo AB. Estimation of total free fatty acid and cholesterol content in some commercial edible oils in Ethiopia, Bahir DAR Journal of Cereals and Oil seeds, 2011; 2(6): 71-6.
13. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants: African Journal of Biotechnology, 2005; 4 (7): 685-8.
14. Harsha N, Sridevi V, ChandanaLakshmi MVV, Rani K, Vani NDS. Phytochemical Analysis of Some Selected Spices, 2013; 2:11.

15. Choia CW, Kima SC, Hwanga SS, Choia BK, Ahna HJ, Leea MY, Parkb SH, Kimc SK. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison: Plant Science, 2002; 163(6): 1161–8.

16. Wojdyłoa A, Oszmiańskia J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs: Food Chemistry, 2007; 105(3): 940–9.

17. Xiaoxi C, Wenhong L. Potent antagonistic activity of newly isolated biological control Bacillus subtilis and novel antibiotic against Erysiphe graminis f.sp tritici: Journal of Medicinal Plants Research, 2011; 5(10): 2011-4.

18. Latha LY, Sasidharan AS, Zuraini Z, Suryani AS, Shirley AL, Sangetha SB and Davaselvia M, Antimicrobial Activities and Toxicity of Crude Extract of the Psophocarpus Tetragonolobus Pods: Afr J Tradit Complement Altern Med. 2007; 4(1): 59–63.

How to Cite: Sharma M, Neerajarani G, Mujeeb CA, Anu V, Sravan B, Kumar A. Antioxidant, Antimicrobial, And Phytochemical Analysis of *Bauhinia Malabarica:* An In-vitro Study. Int J Adv Health Sci 2014; 1(6): 1-13.