

PHARMA SCIENCE MONITOR
AN INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES

**ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF THE BARK
OF *BAUHINIA TOMENTOSA* LINN.**

S. Gopalakrishnan* and E. Vadivel

Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli-627 012, India.

ABSTRACT

The antimicrobial activities of the petroleum ether (40°- 60° C), benzene, chloroform, ethanol and water extracts of the bark of *Bauhinia tomentosa* (Fam, *Fabaceae*) have been evaluated against two gram positive bacteria, *Staphylococcus aureus* and *Enterococcus faecalis*; two gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and two fungi, *Candida albicans*, *Candida tropicalis* by using zone of inhibition method. Ethanolic extract of the bark showed the maximum antimicrobial activity. Hence the minimum inhibitory concentrations (MIC) for the ethanolic extract of the bark of the plant were also determined. The results indicate the efficacy of the plant as a potent antibiotic.

Keywords: *Bauhinia tomentosa*, Antibacterial, Antifungal, Zone of Inhibition, Minimum inhibitory concentration.

INTRODUCTION

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. Several antimicrobial drugs are available now a days, but their use is limited by a number of factors, such as low potency, emergence of resistant strains and drug toxicity. Therefore the search of new more effective antimicrobial agents is necessary^[1,2]. Herbal medicines are in great demand in the developed as well as in developing countries for primary health care because of their wide biological and medicinal activities, higher safety margin and lower costs. *Bauhinia* is a well known plant in herbal medicine for the therapeutic efficacy of its different species. One of the most important species of this genus is *Bauhinia tomentosa* Linn. (Fam, *Fabaceae*). It is commonly known as “Kanjana” in Tamil and “Phalgu” in Sanskrit, The dried leaves, buds and flowers are prescribed in dysentery^[3]. The bruised

bark is applied externally to tumors and wounds. A decoction of the root-bark is administered for inflammation of the liver and it is also used as a vermifuge. An infusion of the bark is also used as an astringent gargle. Leaf has the cytotoxicity and antioxidant activity, Flowers have anti-hyperglycemic and anti-lipidemic activity^[4]. Experimental studies suggest that ethanolic extract of the dried leaf contain kaempferol-7-O-rhamnoside, kaempferol-3-O-glucoside, quercetin-3-O-glucoside and quercetin-3-O-rutinoside^[5].

However no work has been done so far on the antimicrobial activity of the plant. Hence the present study was undertaken to investigate the antimicrobial activity of the petroleum ether (40°- 60° C), benzene, chloroform, ethanol and water extracts of the bark of *Bauhinia tomentosa* on the pathogenic strains of two gram positive bacteria viz., *Staphylococcus aureus* and *Enterococcus faecalis*, two gram negative bacteria viz., *Escherichia coli*, *Pseudomonas aeruginosa* and two fungi, viz. *Candida albicans*, *Candida tropicalis* by using zone of inhibition method. The corresponding solvents are used as solvent control. The standard antibiotics Gentamycin 1µg/disc and ketoconazole 10 µg/disc are used as standard for bacteria and for fungi respectively.

MATERIALS AND METHODS

Plant materials

The plant specimens for the proposed study was collected in the month of September from Courtallam Hills of Tirunelveli District, Tamil Nadu and identified by Prof.P.Jayaraman, Plant Anatomy Research Center, West Thambaram, Chennai.

A voucher specimen (MSU/PHAR/HER-138) was preserved in the Herbarium of the Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli -627 012.

Extraction of plant material

The fresh bark was cleaned with distilled water to remove extraneous matter, shade-dried until to get constant weight and then powdered. The dried powder of the bark of the plant (500 g) was successively extracted using petroleum ether (40°- 60° C), benzene, chloroform, ethanol and water by using Soxhlet apparatus. The last trace of solvent was removed under reduced pressure distillation and then vacuum dried. Finally the dried crude extracts were used for the study.

Organisms used

Bacterial strains used for testing included *Enterococcus faecalis* (NCIM 2276), *Staphylococcus aureus* (NCIM 2079), *Escherichia coli* (NCIM 5033), *Pseudomonas aeruginosa* (NCIM 2036). The fungi used was *Candida albicans* (NCIM 3557), *Candida tropicalis* (NCIM 3573). These were obtained from National Chemical Laboratory, Pune, India. The stock culture was maintained on Mueller Hinton agar medium (Himedia chemicals) at 37° C.

Preparation of the test organisms

The bacterial and fungal cultures were incubated for 24 hrs at 37°C in nutrient agar slants and sabourand glucose slants (Himedia, Mumbai, India) respectively. Before streaking, each culture was diluted (1:10) with fresh sterile nutrients broth. Plates were prepared by pouring 20 ml of freshly prepared No.1 medium (Himedia, Mumbai, India) into 20 mm x 100 mm Petri plates. Inoculum (5 ml) was poured directly over the surface of prepared plates to uniform depth of 4 mm and then allowed to solidify at room temperature.

Antimicrobial assay

The antimicrobial activity was measured by Disc diffusion method^[6]. Test drug solution (1000 µg) of each extract was prepared by dissolving 10 mg of each extract separately in 10 ml of the respective solvents. The test bacterial and fungal strains were inoculated into agar plates (Himedia, Mumbai) separately. Then the sterile disc containing each test drug solution of the plant extract (200 µl) was placed over the seeded agar plates in such a way that there is no overlapping of zone of inhibition. The standard antibiotics Gentamycin 1µg/disc and Ketoconazole 10 µg/disc are used as standard for bacteria and for fungi respectively. The plates were kept at room temperature for two hrs to allow diffusion of the test drug into the agar; they were incubated for 24 and 48 hrs at 37 °C for the bacterial and fungal strains respectively. After the incubation period was over, the plates were observed for zone of inhibition (ZI) measured in millimeters (mm). From the results, the Active Index (AI) and Proportion Index (P.I) were calculated using the following formulas.

$$\text{Active Index (A.I)} = \frac{\text{Inhibition zone of the test sample}}{\text{Inhibition zone of the standard}}$$

$$\text{Proportion index (P.I)} = \frac{\text{Number of positive results obtained for individual extract}}{\text{Total number of tests carried out for each extract}}$$

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the ethanolic extract of *Bauhinia tomentosa* were determined in µg/ml. The samples of the extract were prepared at five different concentrations *viz.* 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 10 µg/ml. The solvent, 90% ethanol was used as a solvent control.

RESULTS AND DISCUSSION

In vitro preliminary screening of the antimicrobial activity of the various extracts of *Bauhinia tomentosa* was studied against the micro-organisms using the filter paper disc diffusion method. The antimicrobial effects of plant extract against the different strains are presented in Table 1. From Table 1, it is very clear that both the ethanol and water extracts inhibited the growth of all tested strains of bacteria and fungus, whereas the benzene extract was effective in the *E. coli* only. But the petroleum ether and the chloroform extracts were resistant to all the tested strains of bacteria and fungus. The ethanol extract showed the maximum activity against all the tested strains used in the present study followed by water extract. The difference in the activity may be due to the different secondary metabolites present in the ethanol and the water extracts.

The ethanol extract of *Bauhinia tomentosa* at the concentration of 1000 µg/ml shows antimicrobial activity on the tested microorganism in the following decreasing order, *P.auriginosa* (23mm), *C.albicans* (21mm), *E.coli* (20mm), *C.tropicalis* (19mm), *S. aureus*(18mm) and *E.faecalis* (16mm) (Table.1). The results were also expressed by means of Active Index (Table.1) and Proportion Index (Fig.1).

The water extract of *Bauhinia tomentosa* at the concentration of 1000 µg/ml shows antimicrobial activity on the tested microorganism in the following decreasing order, *P.auriginosa* (19mm), *E.coli* (14mm), *C.albicans* (13mm), *S. aureus*(12mm), *C.tropicalis* (11mm) and *E.faecalis* (10mm) (Table -1).

The results indicate that the gram negative bacteria, *P.auriginosa* shows the highest activity in both the ethanol and water extracts, The antimicrobial activity of the ethanol extract (23 mm) is higher than that of water extract (19 mm).

TABLE 1: ANTIMICROBIAL ACTIVITY OF THE VARIOUS EXTRACTS OF THE BARK OF *BAUHINIA TOMENTOSA* LINN.

Name of the Organism	Zone of Inhibition (mm)										
	Petroleum ether (40°-60°C)		Benzene		Chloroform		Ethanol		Water		Standards
	DIZ*	AI [#]	DIZ*	AI [#]	DIZ*	AI [#]	DIZ*	AI [#]	DIZ*	AI [#]	
<i>Escherichia coli</i>	-	0	8	0.35	-	0	20	0.86	14	0.61	23 ^a
<i>Pseudomonas aeruginosa</i>	-	0	-	0	-	0	23	0.92	19	0.76	25 ^a
<i>Staphylococcus aureus</i>	-	0	-	0	-	0	18	0.69	12	0.46	26 ^a
<i>Enterococcus faecalis</i>	-	0	-	0	-	0	16	0.80	10	0.50	20 ^a
<i>Candida albicans</i>	-	0	-	0	-	0	21	0.72	13	0.45	29 ^b
<i>Candida tropicalis</i>	-	0	-	0	-	0	19	0.70	11	0.41	27 ^b

*DIZ- Diameter of zone of Inhibition; [#]AI-Active Index,
a – Gentamycin; b - Ketoconazole; - No inhibitory effect.

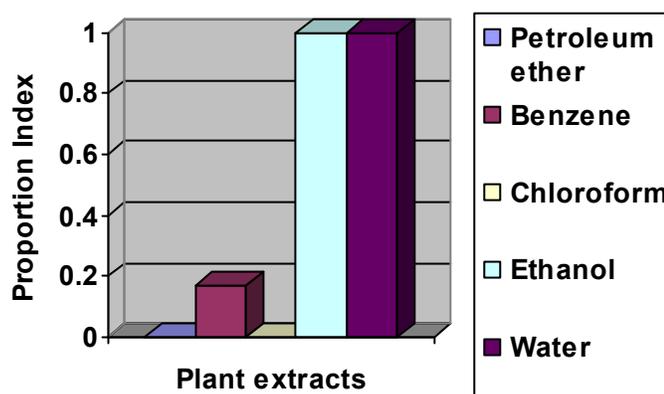


Figure 1

Proportion Index of Antimicrobial activity of the various extracts of the bark of *Bauhinia tomentosa* Linn.

**TABLE 2: MIC OF THE ETHANOLIC EXTRACT OF THE BARK OF
BAUHINIA TOMENTOSA LINN.**

Name of the Organism	Zone of Inhibition (mm)				
	10 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
<i>Escherichia coli</i>	-	08	10	13	16
<i>Pseudomonas aeruginosa</i>	-	09	12	15	20
<i>Staphylococcus aureus</i>	-	-	07	11	13
<i>Enterococcus faecalis</i>	-	-	07	09	12
<i>Candida albicans</i>		08	11	14	18
<i>Candida tropicalis</i>	-	-	08	11	15

- No inhibitory effect.

The minimum inhibitory concentration (MIC) of the ethanolic extract of the bark of *Bauhinia tomentosa* is presented in Table. 2. From the results, it is clear that the MIC value varies from 25 µg/ml and 50 µg/ml. The Gram negative pathogens, *P. aeruginosa* (09mm), *E. coli* (08mm) and the fungus, *C. albicans* (08mm) show MIC value at 25 µg/ml. The Gram positive pathogens *S. aureus* (07mm) and *E. faecalis* (07mm) and the fungus *C. tropicalis* (08mm) shows MIC at 50 µg/ml.

CONCLUSION

The results obtained in this study demonstrated that the medicinal plant, *Bauhinia tomentosa* Linn. displays *in vitro* antimicrobial activity. The high degree of antibacterial activity and antifungal activity seems to confirm the folk therapy of infections and traditional therapeutic claims of this herb. Thus, it has the potential to be used for medicinal purposes or antimicrobial natural additives in cosmetic and food industries or possibly as safe alternatives to synthetic antimicrobial drugs

ACKNOWLEDGEMENT

The authors thank Prof.P.Jayaraman, Plant Anatomy Research Center, West Tambaram, Chennai for the identification of the plant and Dr.S.Ahmed John, Head,

Post Graduate and Research Department Of Botany, Jamal Mohamed College (Autonomous), Tiruchirappalli – 620 020, Tamilnadu for the anti-microbial studies.

REFERENCES

1. Anonymous : The Wealth of India, Raw Material, Vol 11, 55, 1988.
2. Penna C, Marino S, Vivot E, Cruanes M.C, Munoz J.D, Cruanes J, Ferraro G, Gutkind G and Martino V: Antimicrobial activity of Argentina plants used in the treatment of infectious diseases. Isolation of activity compounds from *Sebastiania brasiliensis*. *J.Ethanopharmacol*, 77; 37-40, 2001
3. Quriroga.E.N, Sampietro A.R and Vattuone M.A: Screening antifungal activities of selected medicinal plants, *J Ethanopharmacol*, 74; 89-96, 2010.
4. Mannangatti V, Ayyasamy B, Rangasamy M, Emin B. and Natesan S.K: Anti-hyperglycemic and anti-lipidemic activity of ethanolic extract of *Bauhinia tomentosa* (linn) flower in normal and streptozotocin-induced diabetic rats, *J Global Pharma Technology*, 2(3); 71-76, 2010.
5. Aderogba MA, McGaw LJ, Ogundaini AO and Eloff JN: Cytotoxicity Study of Antioxidant Flavonoids from *Bauhinia tomentosa* leaf Extract, *Nigerian J Natural Products and Medicine* 12; 50-54, 2008.
6. Vanden Berghe, D.A and VlietinckA.J.: Screening methods for antibacterial and antiviral agents from higher plants. In *methods in plant Biochemistry-Assay for Bioactivity*, Academic Press: London, Vol 6, 47-69, 1991.

For Correspondence:

S. Gopalakrishnan

Department of Pharmaceutical Chemistry,

Manonmaniam Sundaranar University, Tirunelveli-627 012, India.

Email: sgkmsu@yahoo.co.in