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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF STEM AND BARK OF "*Bauhinia blakeana*"

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ABSTRACT

Certain bauhinia species are utilized as folk medicines worldwide including Africa, Asia, South America, and also Central America. In recent years, the traditional medicine of India shows great interest in plants because of the presence of biologically active compounds. The *Bauhinia blakeana* is also known as Hong Kong orchid tree, grown in Hong Kong botanical garden from a single ancestor. It has great horticultural value. It is sterile and considered a hybrid origin. The plant belongs to Fabaceae family having potent antibacterial activity against various bacteria. The various parts of *Bauhinia blakeana* tree are popular in various systems of medicine in India for the cure of various diseases. In the present study, the microbial investigation was carried out by the disc diffusion method on the Bark and stem of *Bauhinia blakeana* to find the antibacterial potency present in it. The quantitative investigation revealed that the stem and bark extract of *Bauhinia blakeana* was potent against the selected microbes. The antibacterial activity was tested against both Gram-positive and Gram-negative bacteria. The phytochemical analysis indicates the presence of alkaloids, flavonoids, tannins, saponins, and sterols. We concluded that the presence of secondary metabolites might be the cause of their antibacterial activity.

KEYWORDS: *Bauhinia blakeana*, Disc diffusion, stem & bark, antibacterial, secondary metabolites.

1. INTRODUCTION

Micro-organisms are the most common reason for the cause of infectious diseases. An antibacterial agent is a substance that kills or inhibits the growth of bacteria. In India, numerous infectious diseases controlled by herbal remedies have been proved.[1] Various

species of plants are utilized for treating various diseases. According to WHO, as per the population in India, the use of herbal medicine has been a growing shift in interest towards traditional medicine. The emerging need for the traditional antibacterial was increased to reduce the harm and side-effects produced by the anti-bacterial. The traditional anti-bacterial agents were effective against various infectious diseases and simultaneously it mitigates many side effects which are associated with synthetic antibacterial.[2] *Bauhinia blakeana* belongs to the Family Fabaceae is an evergreen 'Hong Kong Orchid' tree commonly found in India. The tree is well known for its horticultural value. It is considered a hybrid origin. The origin of *Bauhinia blakeana* was reported from the hybridization between the two parenteral species namely Bauhinia purpurea and Bauhinia variegata. *Bauhinia blakeana* Dunn possesses no seeds because it is completely sterile. The sterility is probably due to the hybrid origin.[3] The evidence showed that *Bauhinia blakeana* is not naturally stabilized and is only maintained by artificial propagation. It is generally propagated by grafting onto the rootstocks of other *Bauhinia blakeana* species. Certain Bauhinia Species are utilized as folk medicine worldwide including Asia, Africa, South America, and Central America. It possesses anti-cancer activity, anti-diabetic activity, anti-helmentic activity, insecticidal, anti-inflammatory as well as a wound healing property. There was no report about the antibacterial activity of stem and bark extract of this plant. Hence, in the present study, the methanolic extract of *bauhinia blakeana* was taken for evaluation.[4-6]

2. MATERIALS AND METHODS

2.1. COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

The fresh parts of the plant (stem and bark) were collected from sankari, Salem district, Tamilnadu, India. This plant was authenticated by the Department of Botany, Swami Vivekanandha Arts and Science College for Women, Elayampalayam, Namakkal (Dt). The stem and bark were thoroughly washed and dried under shade for 10-15days. Before analysis, it was segregated, pulverized by a mechanical grinder to a coarse powder.

2.2. SOLVENTS USED FOR EXTRACTION

Chloroform and methanol were used as solvents for the extraction.

2.3. PREPARATION OF CRUDE EXTRACT

The hot percolation method was performed to prepare the chloroform and methanolic extract using a soxhlet extractor.100 grams of stem and bark powdered material of *Bauhinia blakeana* was weighed and by using a cotton bed, the thimble of soxhlet apparatus was

packed. The packed material was individually treated with 500ml of Chloroform & 500ml of methanol. Each setup was proceeded to reflux for 12 hrs at 20-30^{0}C. Then the extract was concentrated by using the evaporation technique until a required state of crude extract was obtained.

2.4. PHYTOCHEMICAL SCREENING

Phytochemical screening of the crude chloroform and methanol extracts of *Bauhinia blakeana* was carried out qualitatively for the presence of alkaloids, flavonoids, tannins, saponins, sterols by using the standard protocols.

2.5. MICROBIAL STRAIN

The microbial strains were collected from the Acme Progen Biotech (India) Pvt. Ltd. Balaji Nagar, Salem as pure cultures and utilized for the evaluation. Few microbial strains were selected according to the Gram-positive and Gram-negative categories. The Gram-positive bacteria namely *Staphylococcus aureus* (MTTCC96), *Bacillus subtilis* (MTTCC121), and the Gram-negative bacteria namely *Escherichia coli* (MTTCC443), *Pseudomonas aeruginosa* (MTTCC424) were taken for the evaluation and were cultured on Muller-Hinton Agar medium at 35 ºC.

2.6. STANDARD ANTIBIOTIC

Tetracycline - 20 microliter/Well (Concentration - 10mg/ml) was used as standard antibiotic against pathogens.

2.7. ANTI-BACTERIAL ASSAY

2.7.1. INOCULUM PREPARATION

The method of inoculum preparation is as follows,

From the Agar plate culture select at least three to five well-isolated colonies of similar morphological types. By using a loop, the top of each colony was touched and the growth was transferred into a tube containing a suitable broth medium such as tryptic soy broth (4 to 5ml). The broth culture is incubated at 35ºC. Monitor the 0.5 McFarland standard (usually 2 to 6 hours) until it achieves or exceeds the turbidity.[7,8]

2.7.2. INOCULATION OF TEST PLATES

Prepare Muller-Hinton agar (MHA) plates. A sterile cotton swab was dipped into the adjusted suspension within 15 minutes after adjusting the turbidity of the inoculum suspension.

Several times the swab should be rotated and above the fluid level, the inside wall of the tube was pressed firmly. The excess inoculum from the swab was removed. The sterile agar surface was entirely streaked by the swab which was inoculated on the dried surface of the Muller-Hinton agar plate. By repeating this procedure more than twice a time, approximately at 60^0C, rotated the plate each time for the confirmation of even inoculum distribution. Finally, the rim of the agar was swabbed. A sterile 6mm filer paper disc was placed on the plate and immediately added the test sample which had already dissolved in water or DMSO. The plates were left for 30 min at room temperature to allow diffusion and were incubated at 35^oC for 24hrs.[9,10]

3. RESULT AND DISCUSSION

3.1. PERCENTAGE YIELD OF THE EXTRACT

The yield of the extract was summarized in table 1 and it reported that the yield of methanol extract was higher than the chloroform extract.

Table 1: Details of soxhlet extraction of stem and bark extract of *"bauhinia blakeana".*

Weight of Plant Material(gm)	Solvent Used	The volume of the solvent(ml)	Sample-solvent ratio[w/v]	Weight of Residue(gm)	Percentage Yield (%)	
100	chloroform	500	1:5		1.46	1.46
100	Methanol	500	1:5		5.32	5.32

3.2. PHYTOCHEMICAL SCREENING

The chloroform and methanol extracts of the stem and bark of *Bauhinia blakeana* were screened for the evaluation of phytochemical groups. The phytochemical test revealed the presence of alkaloids, flavonoids, saponins, tannins, and sterols.[11] The results of the phytochemical screening were given in table 2.

Table 2: Phytochemical Screening.

S. No	Secondary metabolites	Test	Chloroform Extract of Stem & Bark	Methanol Extract of Stem&Bark
1	Alkaloids	Mayer's test	-	-
		Dragendroff's test	++	+
		Wagner's test	-	+
2	Flavonoids	Shinoda test	WP	++++
		Alkali test	++	+
		Lead Acetate test	-	+
3	Saponin	Frothing test	+++	++
4	Tannins	Ferric chloride test	+++	WP

5	Phytosterol	Salkowsik's test	++++	+++
		Libermann – Burchard's test	+	++++

WP = weakly present; +++ rich; ++ moderate**;** + poor**;** - absent of secondary metabolites.

3.3. ANTIBACTERIAL EFFECT OF METHANOLIC EXTRACT OF STEM AND BARK OF *BAUHINIA BLAKEANA*

Table: 3 show the antibacterial effect of *MEBB*. The present study revealed that *MEBB* possesses potential antibacterial activity against all the four strains given below.

TABLE 3.

S.NO	TEST ORGANISM	ZONE OF INHIBITION IN (mm)				
		Std	25 µl	50 µl	75 µl	100 µl
1	*Staphylococcus aureus*	26	11	15	18	21
2	*Pseudomonas aeruginosa*	13	10	14	19	22
3	*Bacillus subtilis*	28	8	12	15	18
4	*Escherichia coli*	30	9	11	14	17

The maximum zone of inhibition was observed against *P.aeruginosa* and *S. aureus* at a maximum tested concentration of 100 µl.

STAPHYLOCOCCUS AUREUS

It was evident from the figure fig-1 while increasing the methanolic extract of stem and bark of *bauhinia blakeana* concentration an increasing zone of inhibition was observed against the growth of human pathogens.

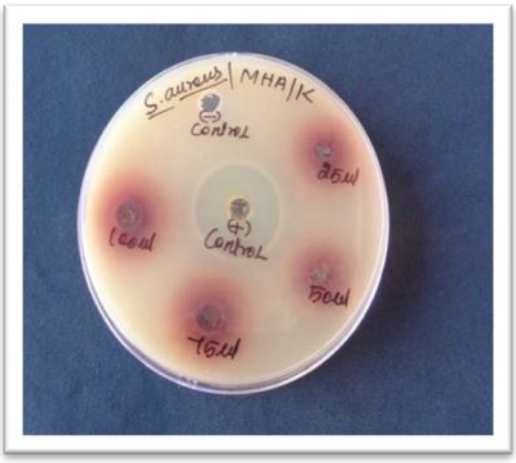
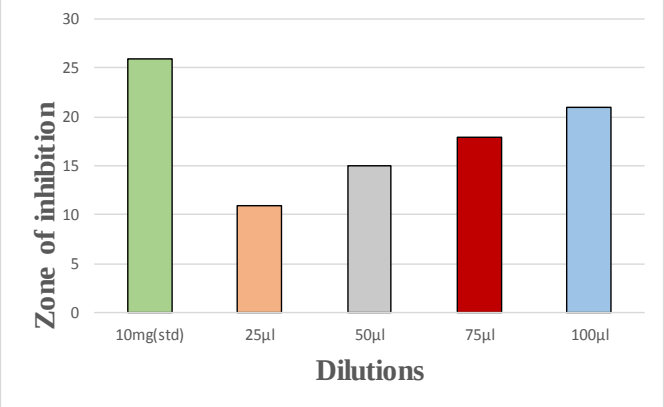


Fig. 1.

EFFECT OF *MEBB* ON ZONE OF INHIBITION *(S.AUREUS)*



Graph: 1.

BACILLUS SUBTILIS

It was evident from the figure fig-2 while increasing the methanolic extract of stem and bark of *bauhinia blakeana* concentration an increasing zone of inhibition was observed against the growth of human pathogens.

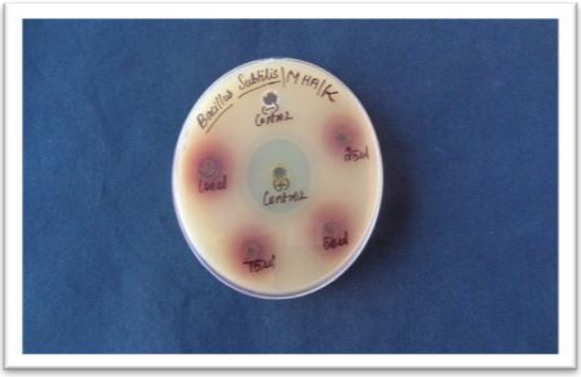
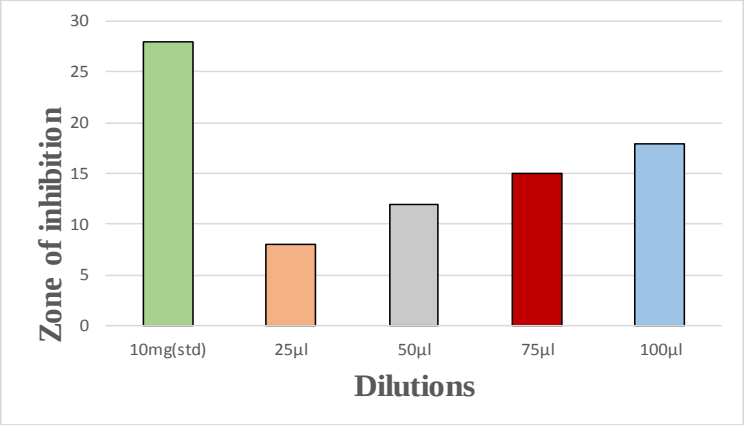


Fig: 2.

EFFECT OF *MEBB* ON ZONE OF INHIBITION *(B. SUBTILIS)*



Graph: 2.

ESCHERICHIA COLI

It was evident from the figure fig-3 while increasing the methanolic extract of stem and bark of *bauhinia blakeana* concentration an increasing zone of inhibition was observed against the growth of human pathogens.

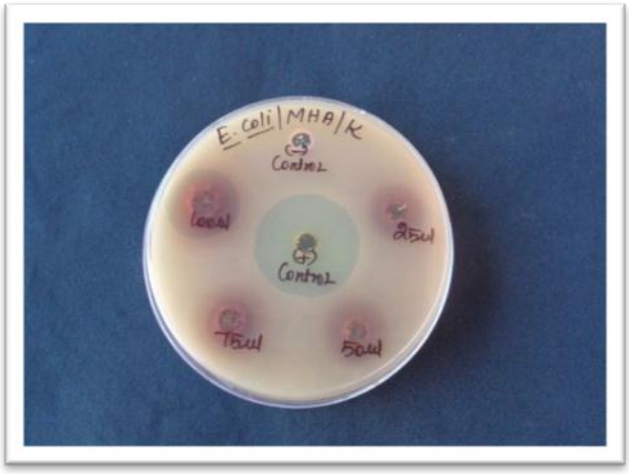
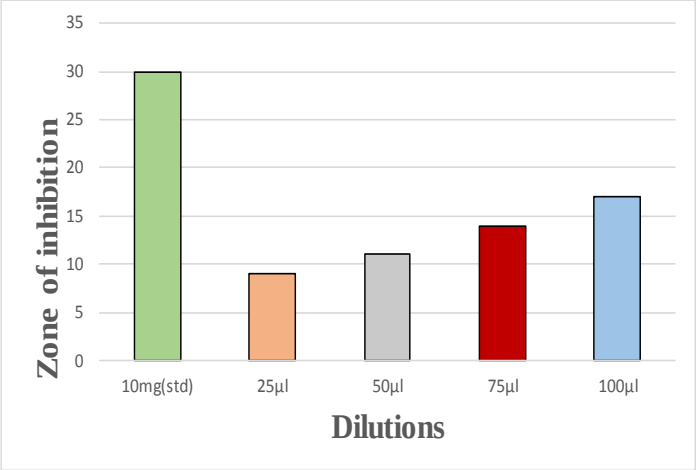


Fig. 3.

EFFECT OF *MEBB* ON ZONE OF INHIBITION (*E.COLI*)



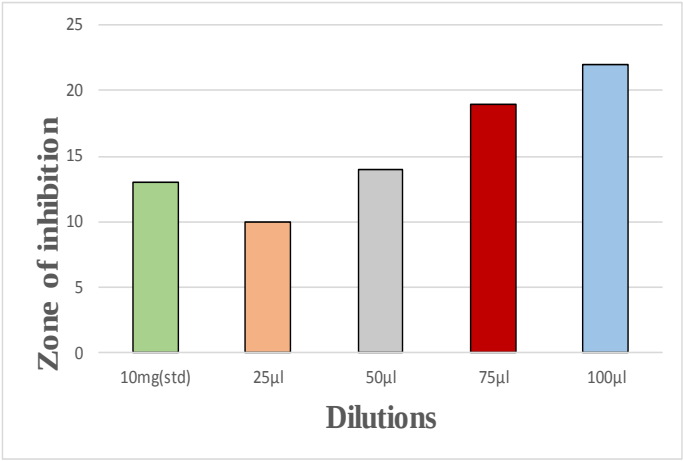
Graph: 3.

PSEUDOMONAS AERUGINOSA

It was evident from the figure fig-while increasing the methanolic extract of stem and bark of *bauhinia blakeana* concentration an increasing zone of inhibition was observed against the growth of human pathogens.

Fig. 4.

EFFECT OF *MEBB* ON ZONE OF INHIBITION*(P.AERUGINOSA)*



Graph: 4.

4. CONCLUSION

The result of this study reveals anti-bacterial activity against test organisms which may be due to the presence of various secondary metabolites in the stem and bark of *Bauhinia blakeana.* The results of this study are very encouraging and indicate that this plant should be more extensively studied to explore its potential in the treatment of many infectious diseases. Further investigation is needed to determine the actual chemical structure of the active compound by using advanced analytical techniques.

5. ACKNOWLEDGEMENT

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