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Evaluation of Anti-microbial Potential of Indian *Bauhinia vahlii* (stembark)

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ABSTRACT

The antimicrobial potency of the stem bark of *Bauhinia vahlii* have been studied using the petroleum ether, benzene, chloroform and ethanol extract against Gram-positive bacteria (Two strains), Gram-negative bacteria (Two strains) and two fungi strains by disc diffusion method. Micro-dilution methods, for the determination of minimal inhibition concentration (MIC) and the minimal bactericidal and fungicidal concentration (MBC, MFC). The ethanol extract at a concentration of 30 to 60 µg/disc and chloroform extract at a concentration 60 µg/disc showed significant activity against all the bacteria and fungus. All the extracts of *Bauhinia vahlii* have got moderate action but chloroform and ethanol extracts have got significant activity against Candida krusei, Candida albicans, Escherichia coli, Salmonella typhi, Staphylococcus aureus, and Bacillus subtilis

Keywords: Antibacterial, antifungal, *Bauhinia vahlii*, minimal inhibition concentration, minimal bactericidal and fungicidal concentration.

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INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine [1, 2]. This plant based traditional medicine system continues to play an essential role in health care, with about 80% of the worlds inhabitants relying mainly on traditional medicines for their primary health care [1, 3]. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy [4, 5]. Recently, scientific interest in medicinal plants has burgeoned due to the increased efficiency of plant derived drugs and raising concern about the side effects of modern medicine. The efficacy of current antimicrobial agents has been reduced due to the continuing emergence of drug resistant organisms and the adaptations by microbial pathogens to commonly used antimicrobials. Therefore, the search for new drugs from plants continues to be a major source of commercial drugs. Plant based antimicrobials represent a vast untapped source of medicines even after their enormous therapeutic potential and effectiveness in the treatment of infectious disease; hence, further exploration of plant antimicrobials needs to occur. The screening of plant extracts and their products for antimicrobial activity has shown that, higher plants represent potential sources of novel antibiotic prototypes [6,7].

Bauhinia vahlii [8] is a gigantic climbing ever green tree and largest creeper in India and can grow up to 10-30 m long. The woody stem can get as thick as 20 cm. The spreading stout branches are covered with rusty fine hair. Branchlets [9] densely pubescent and terminating in a pair of revolute tendrils; young branches, tendrils, petioles, underside of leaves especially along the nerves and inflorescence clothed with dense ferruginous tomentum. Leaves [10] very variable in size, often up to 18 in. diam., as broad as long or broader, deeply cordate, 11-15 nerved, cleft through about 1/3 of the length, sub-coriaceous, dark green and glabrescent above more or less downy beneath; lobes obtuse, rounded; petiole 3-6 in. long, stout.Flowers[10] white, on long slender pedicles, in terminal corymbose or corymbose racemes.

Fruit [11] is a flat woody pod with fine rusty hairs, 20-30 cm long. The plant has been reported to have agathisflavone, flavonoid, betulinic acid, triterpene, campesterol and steroid in leaves [12], catechin, gallic acid methyl ester, benzenoid, mopanol and 4-O-methyl ester[13]. The plant is also reported to contain kaemferol, quercetin, rutin, betulinic acid [14] and Quercitrin, Stigmasterol, Sitosterol[15] in leaves.

The purpose of the present study was to evaluate the antimicrobial potential of *Bauhinia vahlii* stem bark extracts using disc diffusion assay for bacteria and fungi.

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MATERIALS AND METHODS

Materials

Solvents such as Pet.ether, Benzene, Chloroform and Ethanol used were of analytical grade and obtained commercially from Merck- Limited, India, Mumbai.

Collection and identification of plant material

The Plant *Bauhinia vahlii* were collected in the month of October from Padiabahal, Sambalpur, Odisha , India. The plant material was taxonomically identified by Dr. (Mrs.) Uma Devi, Head, Department of Botany, Govt. Women's College, Sambalpur, Odisha. A voucher specimen (GWC/B-315/09) has been deposited in the Herbarium of the Department of School of Pharmaceutical Education & Research, Berhampur University, Berhampur-760007, India for future reference.

Preparation of plant material

The whole plant was first sun dried for several weeks, crushed by hands and dried again. Then the crushed parts of the plant were ground into coarse powder with the help of a mechanical grinder. By using the concept of the nature of solubility and distribution of the active ingredients, powdered material (1400gm) was packed in soxhlet apparatus [16] and extracted successively with Pet. Ether (60-80) to defat the drug; petroleum ether was removed from the powdered defatted drug which was then extracted with benzene, chloroform and 95% of Ethanol as increasing polarity. The whole each mixture then underwent filtration through what man filter paper. The filtrates (Pet. Ether, Benzene, Chloroform and Ethanol filtrate) obtained were evaporated by rotary evaporator at 5 to 6 rpm and at 40ºc temperature. It rendered a gummy concentrates. The gummy concentrate was designated as crude extract which was then freeze dried and preserved at 4^{o}_{C}.

Phytochemical Screening

Qualitative phytochemical tests for the identification of alkaloids, flavonoids, steroids, glycosides, saponins, tannins and terpenoids were carried out for all the extracts by standard procedure [17-20]**.** (Table10)

Microbial cultures:

The following microorganisms were used to test the activity of the extracts. Pure isolates of two Gram positive bacteria such as Escherichia coli (MTCC-443), Salmonella typhi and two Gram negative bacteria such as Staphylococcus aureus (MTCC-1430), Bacillus subtilis

(MTCC-441) and fungi Candida albicans (MTCC-183), Candida krusei were collected from Dept. of Life Science, Sambalpur University,Odisha,India.

Culture media

Nutrient broth, nutrient agar, Sabouraud's dextrose agar purchage from Himedia

Reference antibiotics

Amoxycillin and Miconazole were used as reference antibiotics (RA) against bacteria and yeast, respectively. This was collected as standard sample from Medico Remedies Pvt. Ltd, Maharashtra, India.

Inoculum Preparation

Nutrient broth and Sabouraud dextrose agar (SDA) were used for growing and diluting the microorganism suspensions. Inoculums from bacterial cultures were prepared by picking colonies from 24 h old cultures. Colonies were suspended in 5 ml of a solution containing 0.145 mol of saline per liter. The density was adjusted by spectrophotometer to that of a 0.5 McFarland standard at a wavelength of 530 nm to yield a stock suspension of 1×10^6 to 5×10^6 cells per ml. Two different inoculums sizes were evaluated: 1×10^6 to 5×10^6 cells per ml and a 1:100 dilution containing 1×10^4 to 5×10^4 cells per ml. Plates were swabbed in three directions. Fungal cultures were aseptically inoculated on petri dishes containing autoclaved, cooled, and settled SDA medium. The petri dishes were incubated at 31^0C for 48 h to give white round colonies against a yellowish background. These were aseptically subcultured on SDA slants. The yeast colonies from SDA slants were suspended in sterilized 0.9% sodium chloride solution (normal saline), which was compared with McFarland solution. According to the manufacturer's directions, 1 ml of yeast suspension in normal saline was added to 74 ml of sterile medium and kept at 45°C to give a concentration of 2 x 107 cells/ml.

Determination of MIC:

Double strength nutrient agar was prepared in 500 ml of distilled water which was swirled and mixed thoroughly by heating to allow uniform dissolution, after which 5 ml of it was dispensed into universal bottles and sterilized in an autoclave at 121^oC for 15 min. The agar was allowed to cool to 45^oC and each graded solution was then mixed gently with molten double strength nutrient agar in a petri dish and allowed to solidify for one hour. Extracts' concentrations of 100, 90, 80, 70, 60, 50, 40, 30, 20, 10 and 5 µg/ml [21] respectively were prepared by serial dilution. Each plate was divided into six equal sections and labeled accordingly to correspond to six test organisms. Two 6 mm diameter paper discs (Whitman No.1) were placed aseptically into each labeled section of the plate using sterilized forceps. With an automatic micropipette, 20 µl of each bacterial suspension was taken and transferred

aseptically and carefully into each appropriate pre-labeled paper disc on the agar plates. The plates were incubated for 24 h at 37^{o}C after which they were observed for growths or death of the test organisms. In contrast, fungal cultures were incubated at 31 °C for 48 h in SDA medium. The lowest concentration inhibiting growth was taken as the minimum inhibitory concentration (MIC). The average of 3 values was calculated and that was the MIC for the test material. (Table 1 to 4)

Determination of MBC and MFC:

This was carried out to know if the organisms could be killed completely or their growths could only be inhibited. Another set of plates of nutrient agar were prepared and sterilized in an autoclave as earlier described. The paper discs in all the plates from the MIC tests were re-activated. Emphasis was mostly paid to the MIC plates and the preceding plates. The re-activation was done in a mixture of 0.5% egg lecithin and 3% Tween 80 solution in a test tube. The reactivated organisms were sub-cultured into appropriately labeled quadrants of the sterilized nutrient agar plates using wire loop into each test tube and streaking uniformly on the labeled quadrants. This was then incubated for 24 h at 37^{o}C after which they were observed for growths [21, 22]. In contrast, fungal cultures were incubated at 31 °C for 48 h in SDA medium. The MBC was the quadrant with the lowest concentration of the extract without growth. (Table 5 to 8)

Disc diffusion assay:

Stock solutions of different extracts of *B.vahlii* were prepared separately by initially dissolving 0.05 g of the extract in 0.5 ml of DMSO to obtain a stock solution of concentration 100 mg/ml. From this stock solution, concentrations of 1 mg/ml were prepared by serial dilution. From the above stock solution further dilutions were made to get 10, 20, 30, 40, 60 µg/ml working solutions. The cork and bore diffusion method of Bauer et al. (1966) and Barry and Thornsberry (1985) were used in the antimicrobial screening. Nutrient agar was inoculated with a microbial cell suspension (200 µl in 20 ml of medium) and poured into sterile petridishes followed by cross-streaking with the same wire loop to achieve uniform spread on the plate. Sterile filter paper discs 6 mm in diameter were impregnated with 20 µl of each extract concentration (30 and 60 µg/ml), which were prepared using the same solvents employed to dissolve the plant extracts, and placed on the inoculated agar surface. Standard 6 mm discs containing rifampicin 30 µg/disc was used as positive control. Negative controls were made using paper discs loaded with 20 µl of the solvents. After pre-incubation for 2 h in a refrigerator the plates were incubated overnight at 37 °C for 18-24 h. In contrast, fungal cultures were incubated at 31°C for 48 h in SDA medium using miconazole 10 µg/disc [23]. Negative controls were made using paper discs loaded with 20 µl of the solvents. At the end of the incubation period antimicrobial activity was evaluated by measuring the zones of inhibition.

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RESULTS AND DISCUSSION

Antimicrobial studies are conducted to investigate the antimicrobial potency of *Bauhinia vahlii* extracts against a few selected strains of bacteria and fungi. Before performing the antimicrobial screening it was important to check the minimum inhibitory concentration (MIC) for bacteria (Table 1 to 4). The determination of MBC (Table 5 to 8) and MFC (Table 5 to 8) were carried out to check whether the organisms could be killed completely or their growths only be inhibited. The ethanol and chloroform extract of *Bauhinia vahlii* exhibited significant activity against all the tested bacteria and fungi. (Table 9).

Table 1: MIC of the Pet. Ether extract of *B.vahlii* some pathogens (bacteria and fungi).

Plant	Test organisms	Concentration (µg/ml) of pet. ether extract of *Bahunia vahlii*										
		100	90	80	70	60	50	40	30	20	10	5
B.vahlii	Escherichia coli	-	-	-	-	-	-	-	-	-*	++	+++
	Salmonella typhi	-	-	-	-	-	-	-	-*	+	++	+++
	Staphylococcus aureus	-	-	-	-	-	-	-	-*	+	++	+++
	Bacillus subtilis	-	-	-	-	-	-	-	-	-*	++	++
	Candida albicans	-	-	-	-	-	-	-	-*	+	++	+++
	Candida krusei	-	-	-	-	-	-	-	-*	+	++	+++

* = MIC concentration, - = No growth, + = little growth. ++ = Adequate growth, +++ = Excess growth

Table 2: MIC of the Benzene extract of *B.vahlii* some pathogens (bacteria and fungi).

Plant	Test organisms	Concentration (µg/ml) of Benzene extract of *Bahunia vahlii*										
		100	90	80	70	60	50	40	30	20	10	5
	Candida krusei	-	-	-	-	-*	+	++	++	+++	+++	+++
B.vahlii	Escherichia coli	-	-	-	-	-	-	-*	+	++	++	+++
	Salmonella typhi	-	-	-	-	-	-	-*	++	+	++	+++
	Staphylococcus aureus	-	-	-	-	-	-	-	-	-*	++	+++
	Bacillus subtilis	-	-	-	-	-	-	-	-	-*	++	++
	Candida albicans	-	-	-	-	-*	+	+	++	+++	+++	+++
	Candida krusei	-	-	-	-		-*	++	++	+++	+++	+++

* = MIC concentration, - = No growth, + = Little growth. ++ = Adequate growth, +++ = Excess growt

Table 3: MIC of the Chloroform extract of *B.vahlii* some pathogens (bacteria and fungi).

Plant	Test organisms	Concentration (µg/ml) of Chloroform extract of *Bahunia vahlii*										
		100	90	80	70	60	50	40	30	20	10	5
B.vahlii	Escherichia coli	-	-	-	-	-	-*	+	++	++	++	+++
	Salmonella typhi	-	-	-	-	-	-	-*	+	++	++	+++
	Staphylococcus aureus	-	-	-	-	-	-	-	-	-*	++	++
	Bacillus subtilis	-	-	-	-	-*	+	++	++	+++	+++	+++
	Candida albicans	-	-	-	-	-	-	-*	+	++	++	+++
	Candida krusei	-	-	-	-	-	-	-	-	-*	+	++

* = MIC concentration, - = No growth, + = Little growth. ++ = Adequate growth, +++ = Excess growth

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Table 4: MIC of the Ethanolic extract of *B.vahlii* some pathogens (bacteria and Fungi).

Plant	Test organisms	Concentration (µg/ml) of Ethanol extract of *Bahunia vahlii*										
		100	90	80	70	60	50	40	30	20	10	5
B.vahlii	Escherichia coli	-	-	-	-	-	-*	+	++	++	+++	+++
	Salmonella typhi	-	-	-	-	-	-*	+	++	++	++	+++
	Staphylococcus aureus	-	-	-	-	-	-	-*	+	++	++	+++
	Bacillus subtilis	-	-	-	-	-*	+	+	++	++	+++	+++
	Candida albicans	-	-	-	-	-	-	-	-	-*	+	++
	Candida krusei	-	-	-	-	-	-	-	-	-*	+	++

* = MIC concentration, - = No growth, + = Little growth. ++ = Adequate growth, +++ = Excess growth

Table 5: MBC and MFC of the pet. ether extract of *B.vahlii* some pathogens (bacteria and fungi).

Plant	Test organisms	Concentration (µg/ml) of pet. ether extract of *Bahunia vahlii*										
		100	90	80	70	60	50	40	30	20	10	5
B.vahlii	Escherichia coli	-	-	-	-	-	-*	+	++	++	+++	+++
	Salmonella typhi	-	-	-	-	-*	+	++	++	+++	+++	+++
	Staphylococcus aureus	-	-	-	-	-	-*	+	++	+++	+++	+++
	Bacillus subtilis	-	-	-	-	-*	+	+	++	++	+++	+++
	Candida albicans	-	-	-	-	-	-	-*	+	++	+++	+++
	Candida krusei	-	-	-	-	-	-*	+	++	+++	+++	+++

* = MBC and MFC concentration, - = No growth, + = Little growth, ++ = Adequate growth, +++ = Excess growth

Table 6: MBC and MFC of the Benzene extract of *B.vahlii* on some pathogens (bacteria and fungi).

Plant	Test organisms	Concentration (µg/ml) of Benzene extract of *Bahunia vahlii*										
		100	90	80	70	60	50	40	30	20	10	5
B.vahlii	Escherichia coli	-	-	-	-	-	-*	+	++	++	+++	+++
	Salmonella typhi	-	-	-	-	-*	+	++	++	+++	+++	+++
	Staphylococcus aureus	-	-	-	-	-	-*	+	++	+++	+++	+++
	Bacillus subtilis	-	-	-	-	-*	+	+	++	++	+++	+++
	Candida albicans	-	-	-	-	-*	+	+	++	++	+++	+++
	Candida krusei	-	-	-	-	-	-*	+	++	+++	+++	+++

* = MBC and MFC concentration,- = No growth,+ = Little growth,++ = Adequate growth,+++ = Excess growth.

Table 7: MBC and MFC of the Chloroform extract of *B.vahlii* some pathogens (bacteria and fungi).

Plant	Test organisms	Concentration (µg/ml) of Chloroform extract of *Bahunia vahlii*										
		100	90	80	70	60	50	40	30	20	10	5
B.vahlii	Escherichia coli	-	-	-	-	-	-*	+	++	++	+++	+++
	Salmonella typhi	-	-	-	-	-*	+	++	++	+++	+++	+++
	Staphylococcus aureus	-	-	-	-	-	-*	+	++	+++	+++	+++
	Bacillus subtilis	-	-	-	-	-*	+	+	++	++	+++	+++
	Candida albicans	-	-	-	-	-	-	-*	+	++	++	+++
	Candida krusei	-	-	-	-	-	-*	+	+	++	++	+++

* = MBC and MFC concentration,- = No growth,+ = Little growth,++ = Adequate growth,+++ = Excess growth

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Table 8: MBC and MFC of the Ethanolic extract of *B.vahlii* some pathogens (bacteria and fungi).

Plant	Test organisms	Concentration (µg/ml) of Ethanol extract of *Bahunia vahlii*										
		100	90	80	70	60	50	40	30	20	10	5
B.vahlii	Escherichia coli	-	-	-	-	-	-	-*	+	++	+++	+++
	Salmonella typhi	-	-	-	-	-	-	-*	+	++	+++	+++
	Staphylococcus aureus	-	-	-	-	-	-	-*	+	++	+++	+++
	Bacillus subtilis	-	-	-	-	-	-*	+	++	++	+++	+++
	Candida albicans	-	-	-	-	-	-	-*	+	++	+++	+++
	Candida krusei	-	-	-	-	-	-	-	-*	+	++	+++

* = MBC and MFC concentration, - = No growth, + = Little growth, ++ = Adequate growth, +++ = Excess growth

Table 9: Antibacterial and antifungal activity of *B. vahlii* (stem bark).

Microorganisms	**Pet.ether (µg/disc)**		**Benzene µg/disc)**		**Chloroform (µg/disc)**		**Ethanol (µg/disc)**		**Amoxycillin (µg/disc)**	**Miconazole (µg/disc)**
	30	60	30	60	30	60	30	60	30	10
Bacteria										
Escherichia coli	+	+	-	-	-	+	++	+++	+++	NT
Salmonella typhi	-	-	-	-	-	+	++	+++	+++	NT
Staphylococcus aureus	-	+	-	+	+	++	++	+++	+++	NT
Bacillus subtilis	-	+	+	+	-	++	+	++	+++	NT
Fungi										
Candida krusei	+	+	-	-	+	+	++	+++	NT	+++
Candida albicans	+	+	-	-	+	++	+++	+++	NT	+++

Experiments were done in triplicate. Disc diameter = 4mm.
Diameter of zone of inhibition: - < 4; + = 5-10; ++ = 11-15; +++ > 16; NT = not tested. DMF had not shown any antimicrobial activity against the tested organisms.

Table 10: Preliminary Phytochemical Investigation of various extracts and powder of *B. vahlii*

Test	**Inference**				
	PD	**PEE**	**BE**	**CE**	**EE**
Test for Carbohydrates	-	-	-	-	-
Test for Gums and Mucilages	+	-	-	-	-
Test for Proteins and Amino Acid	+	-	-	-	-
Test for Fixed Oils and Fats	+	+	+	+	+
Test for Phytosterols	+	+	+	+	+
Test for Glycosides	-	-	-	+	-
Tests for Saponins	+	-	-	-	+
Tests for Flavonoids	+	+	_	+	+
Tests for Alkaloids	+	-	-	-	+
Tests for Tannins and Phenolic Compounds	-	+	+	-	+

The zones of inhibition ranged from 4-20 mm for different extracts against bacteria and fungi. The alcoholic and chloroform extract had promising MIC values against all tested bacteria

and fungi. The resistance of bacteria and fungi towards different drugs can be due to modification of the target site, by pass of pathways, decreased uptake (reduced intracellular concentration of the antimicrobial agent, either reducing membrane permeability or by active efflux pump), enzymatic inactivation or modification of the drug, or over production of the target [24]. The antimicrobial effects of *Bauhinia vahlii* may be attributed to various photochemical contained in its extracts, such as phytosterols, alkaloid, tannins, phenolic compounds and flavonoids (Table 10).

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REFERENCES

[1] Owoabi J, Omogbai E K I , Obasuyi O. Afr J Biotechnol 2007; 6: 882-885.
[2] Cragg GM, Newman DJ. NY Acad Sci 2001; 953: 3-25.
[3] Farnsworth N R, Akerele O, Bingel A S. Bull WHO 1985; 63: 965-981.
[4] Doughari JH, El-mahmood AM, Tyoyina I. African J Pharm Pharmacol 2008; 2(1): 007-013.
[5] Nascimento GGF, Lacatelli J, Freitas PC, Silva GL. Braz J Microbiol 2000; 31: 886- 891.
[6] Aliero AA, Afolayan AJ. African J Biotechnol 2006; 5 (4): 369-372.
[7] Afolayan A. J Pharm Biol 2003; 41: 22-25.
[8] Baker in Hook f. Fl Brit Ind 1876; 2:279.
[9] Prodr. Fl Pen Ind 1834; 297.
[10] Parker. Fl Punj 1956; 3: 180.
[11] Ali P. Acta Biol 1967; 8: 241.
[12] Sultana S, Ilyas M, Kamil M, Shaida WA. J Indian Chem Soc 1985; 62(4):337-338.
[13] Jagdish kR, Krupadanam GLD, Srimannarayana G. Fitoterapia 1990; 61(5): 475-476.
[14] Duret S, Paris RR. Plant Med Phytother 1977; 11:213.
[15] Sultana S, Ilyas M, Kamil M, Shaida W A. J Indian Chem Soc 1985; 62(4): 337-338.
[16] Harborne JB. Phytochemical Methods, A Guide to Modern Techniques of Plant `Analysis. New Delhi: Springer, Rajkamal Electric Press, 1998, 3: 1-32, 40-227.
[17] Shah BN, Nayak BS. Experimental Pharmacognosy. Jalandhar, India: Vikash & Co 2008; 190-200.
[18] Shah CS, Quadry JS. A Text Book of Pharmacognosy. Ahmedabad, India, B.S. Shah Prakashan; 2007-08, 13: 83-86, 97.
[19] Ali M, Text Book of Pharmacognosy, CBS Publishers and Distributers, New Delhi, 2003, 2nd Edn. p. 64-65, 96-97,139-40,272,283.
[20] Tailang M , Sharma A, Phytochemistry ,Theory & Practical, New Delhi,India:Birla Publications; 2008, Ist Edn.p.199.
[21] Ndukwe IG, Habila JD, Bello IA, Okoh JI. Afr J Biotechnol 2006; 5: 1789-91.

[22] Navarro V, Villarial ML, Rojas G, Lozoya X. J Ethnopharmacol 1966; 53:143-47.
[23] Sur-Altiner D, Gürkan E, Mutlu G, Tuzlaci E, Ang O. Fitoterapia 1999;70:187-9.
[24] Navarro V, Villarreal M, Rojas G, Lozoya X. J Ethnopharmacol 1998; 53(3): 143.