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IN-VITRO EVALUATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF MEDICINAL PLANT *BAUHINIA VAHLII*

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Keywords:

Disc diffusion method, Zones of inhibition, *Bauhinia vahlii*, Stem, Leaves

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ABSTRACT: The present study evaluated the antibacterial and antifungal activities of the medicinal plant *Bauhinia vahlii*. The antibacterial and antifungal activities of vacuum dried ethanol extract of the stem and leaves of *Bauhinia vahlii* (EEBVS and EEBVL) were performed using the Disc diffusion method. The antibacterial and antifungal activities recorded significant effects against five bacterial strains, including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and two fungal strains, including *Candida albicans* and *Aspergillus niger*. The ethanol extracts of *Bauhinia vahlii* stem and leaves were showed the significant antibacterial effect of different bacterial strains and antifungal activity of fungal strains in the ethanol extract of stem (14 mm) but more activity observed in the ethanol extract of leaves (17 mm) in the *Escherichia coli* bacterial strain against different bacterial strains as compared to standard drug Amikacin (19 mm) whereas more potent in ethanol extracts of leaves (19 mm) in the fungal strain *Candida albicans* as compared to standard drug fluconazole (21 mm) as observed by zones of inhibitions (ZOI). Therefore, the EEBVL was found to have more potent antifungal activities of the fungal strain against the bacterial strain as observed by zones of inhibitions.

INTRODUCTION: *Bauhinia vahlii* Wight & Arn belong to the family Caesalpiniaceae. It is known as "Maljan" in Hindi [1]. The plant is a giant climbing shrub distributed in the Himalayan region up to 3,000 m above sea level and is also found in Assam, Central India, Bihar, and forest areas throughout India [2, 3]. The leaves of *Bauhinia vahlii* are traditionally used as demulcent, antimicrobial and antidiarrhoeal properties. The stem of this plant is useful for fever, skin disease, and antidysentery agents [4, 5].

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The major secondary constituents of leaves and stem of Bauhinia vahlii contain agathisflavone, betulinic acid, campesterol, flavanol glycoside, kaempferol, quercetin, isoquercitrin, rutoside and β-sitosterol [6, 7]. Hence, in the present study, the vacuum-dried ethanol extracts of leaves and stem of *Bauhinia vahlii* were evaluated for their antibacterial and antifungal activities using the Disc diffusion method [8, 9, 10].

Antimicrobial properties of medicinal plants are microorganisms progressively more reported from different parts of the world [11]. The WHO estimates that plants of their active constituents are used as folk medicine in traditional therapy for 80% of the world population [12, 13]. The harmful microorganisms may be controlled with medicine and this results in the observation of multiple drug-

resistant bacteria and it has created an alarming clinical situation in the treatment of infections. The pharmacological industries have provided several new antibiotics; resistance to these drugs by microorganisms has increased [14, 15]. In general, bacteria have the genetic facility to transmit and acquire resistance to synthetic medicine utilized as therapeutic agents [16]. Natural products from various higher plants can provide a new source of antimicrobial agents with maybe novel mechanisms of action [17]. The plants are rich sources of a wide range of secondary metabolites such as alkaloids, glycosides, flavonoids, tannins and terpenoids, *etc*. found *in-vitro* antimicrobial [18].

FIG. 1: PLANT OF *BAUHINIA VAHLII*

MATERIALS AND METHODS:

Plant Material: *Bauhinia vahlii* stem and leaves were collected from an area of the Chandi Devi Temple placed at Haridwar (Uttarakhand), India. Dr. S. K. Sinha, Scientist-E, Botanical Survey of India, Allahabad authenticated the specimen of the plant. A voucher specimen (BSI/CRC/2021-16/603) of the plant specimen was accumulated in the herbarium department for further knowledge.

Preparation of Extract: The stem and leaves of the *Bauhinia vahlii* of air dried coarsely powdered has sieved by 40 mesh [19] and they were extracted with ethanol (95%) by hot percolation process separately with soxhlet apparatus for two days at 45-50ºC temperature [20, 21]. The extracts were filtered and filtrate distilled at low temperature (55-60ºC) and finally evaporated under reduced vacuum pressure to acquire dried ethanol extract of *Bauhinia vahlii* stem (EEBVS) and ethanol extract of *Bauhinia vahlii* leaves (EEBVL).

Antibacterial and Antifungal Activities: The antibacterial and antifungal activities of ethanol extracts of the *Bauhinia vahlii* stem and leaves (EEBVS and EEBVL) were performed using the Disc diffusion method [22].

Disc Diffusion Method: The media were prepared and autoclaved at 121^{o}C, 15 lbs/inch^{2} pressure for 15 minutes. These media were poured into plates and allowed to solidify. On the surface of the media, the microbial suspension was spread with the help of a sterilized bent shape glass rod. The above-said prepared nutrient agar media was taken in a pre-sterilized Petri dish, and the microorganisms were grown.

The disc of 7 mm was saturated with 20 µl of 5 mg/ml, the concentration of the applied solution on the disc was 100 mg/disc of extract solution of ethanol extract of the *Bauhinia vahlii* stem and leaves, and the disc of standard Amikacin (30 µg/disc) was placed in the center for antibacterial activity and Fluconazole (10 µg/disc) for antifungal activity in each disc. The plates were kept at room temperature for one hour to allow the diffusion of test compounds and then incubated at 37± 0.50C for antibacterial activity for 24 hrs and antifungal activity for 48 hrs, respectively. The diameters of the zone of inhibition in mm were measured and compared with the standard drugs [23].

Preparation of the Solutions of Extract: The ethanol extracts of the *Bauhinia vahlii* stem and leaves, accurately weighed 5 mg of each, were transferred into different10 ml volumetric flasks. These extracts were dissolved into DMSO, and the volume in each flask was made up to 10 ml with DMSO. The concentrations of stock solutions were 5 mg/ml prepared. Now from this stock solution 20 ml was taken and sterilized in the empty disc. Thus, the concentration of the applied solution on the disc was 100 mg/disc in each Petri dish [24].

Bacterial and Fungal Strains: In this study total of five bacterial strains, including two-gram +ve as *Staphylococcus aureus* (ATCC 25923) & *Bacillus subtilis* (ATCC 10774) and three-gram -ve as *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) & *Salmonella typhi* (ATCC 733) and two fungal strains *Candida albicans* (ATCC 10231) & *Aspergillus niger* (ATCC 16404) were used for the assessment of antibacterial and antifungal activities [22, 24].

Preparation of Nutrient Agar Media (2%, pH 6.8±0.2) for Antibacterial Activity: Take about 5 gm beef extract, 5 gm peptone and 2.5 gm sodium chloride dissolved in 400 ml of distilled water in a 500 ml volumetric flask and warmed it. 10 gm of agar dissolved in 50 ml of warm distilled water. The two solutions were mixed and the volume in the volumetric flask was made up to 500 ml of warm distilled water. These nutrient agar media were sterilized in an autoclave at121^{o}C, 15 lb /inch^{2} pressure for 15 min [22, 25].

Preparation of Sabouraud Dextrose Agar Media (2%, pH 6.8±0.2) for Antifungal Activity: To prepare the Sabouraud dextrose agar media, take the accurate weight of the 20 gm dextrose monohydrate and 5 gm peptone and dissolved it in 400 ml of distilled water in 500 ml volumetric flask, and warm. Dissolve 7.5 g of agar in 50 ml of warm distilled water. The two solutions were mixed together, making the volume in a volumetric flask up to 500 ml with warm distilled water. This media was sterilized in an autoclave at 121^{o}C, 15 lbs/inch^{2} pressure for 15 minutes [22, 26].

Preparation of Inoculums: The vial containing lactose dilution (dehydrated powder) of inoculums of five bacterial strains *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* and fungal two strains *Candida albicans* and *Aspergillus niger*, were prepared individually broken using a sterile scalpel knife in an aseptic condition in a conical flask containing 100 ml of nutrient broth. This flask was incubated for 24 hrs at 37° C in the Biological Oxygen Demand (BOD) incubator. After 24 h, a turbid solution was obtained [27, 28].

RESULT AND DISCUSSION: The ethanol extracts of *Bauhinia vahlii* stem and leaves were showed a significant antibacterial effect in the ethanol extract of the stem (14 mm) but more activity observed in the ethanol extract of leaves (17 mm) in the *Escherichia coli* bacterial strain against different bacterial strains as compared to standard drug Amikacin (19 mm) **Table 1** and **Fig. 2** and **Fig. 3**, whereas more potent in ethanol extracts of leaves (19 mm) in the fungal strain *Candida albicans* as compared to standard drug fluconazole (21mm) **Table 2** and **Fig. 4, 5** as observed by zones of inhibitions (ZOI). The possible mechanism of antibacterial activities is shown in **Fig. 6**, which shows three possible mechanisms for antibacterial activities, *i.e.*, cell wall synthesis, irreversible binding with the DNA bases, and penetration into the cell membrane.

TABLE 1: ZONE OF INHIBITION OF ETHANOL EXTRACTS OF *BAUHINIA VAHLII* STEM AND LEAVES ON DIFFERENT BACTERIAL STRAINS

Test Organism	Zone of Inhibition in mm		Amikacin (30 µg/disc)
	Ethanol Extracts (100 µg/disc)		
	Stem Extract (EEBVS)	Leaves Extract (EEBVL)	
Staphylococcus aureus	12	16	18
Bacillus subtilis	11	14	16
Escherichia coli	14	17	19
Pseudomonas aeruginosa	12	15	17
Salmonella typhi	10	12	15

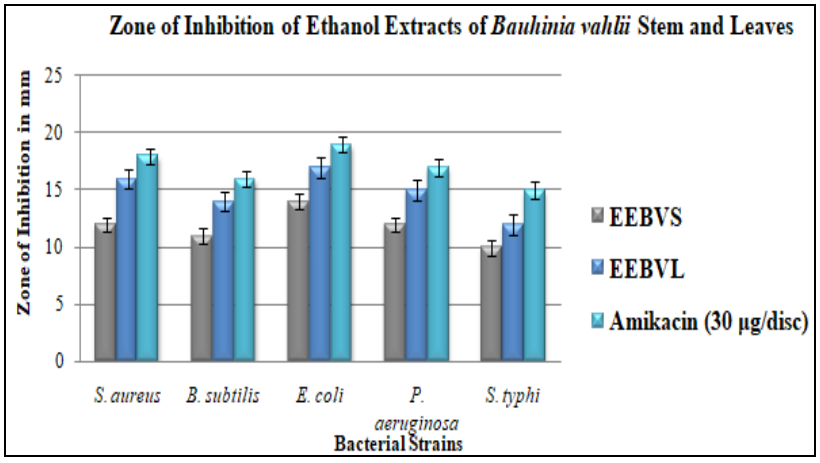

FIG. 2: ZONE OF INHIBITION OF EEBVS, EEBVL AND STANDARD DRUG ON THE BACTERIAL STRAINS

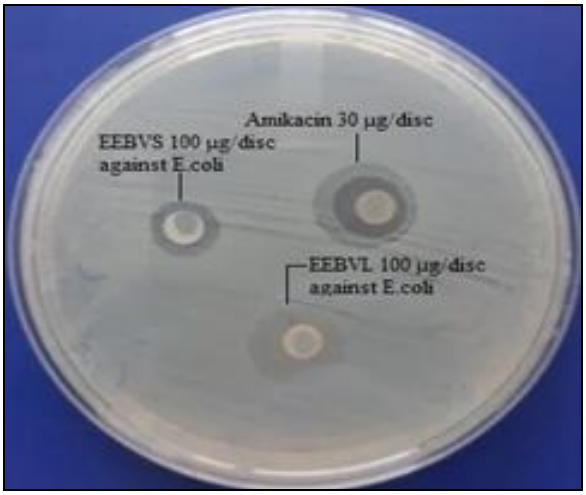

FIG. 3: ANTIBACTERIAL ACTIVITY OF EEBVS, EEBVL AND STANDARD DRUG IN THE *ESCHERICHIA COLI*

TABLE 2: ZONE OF INHIBITION OF ETHANOL EXTRACTS OF *BAUHINIA VAHLII* STEM AND LEAVES ON DIFFERENT FUNGAL STRAINS

Test Organism	Zone of Inhibition in mm		Fluconazole (10 µg/disc)
	Ethanol Extracts (100 µg/disc)		
	Stem Extract (EEBVS)	Leaves Extract (EEBVL)	
Candida albicans	14	19	21
Aspergillus niger	12	17	19

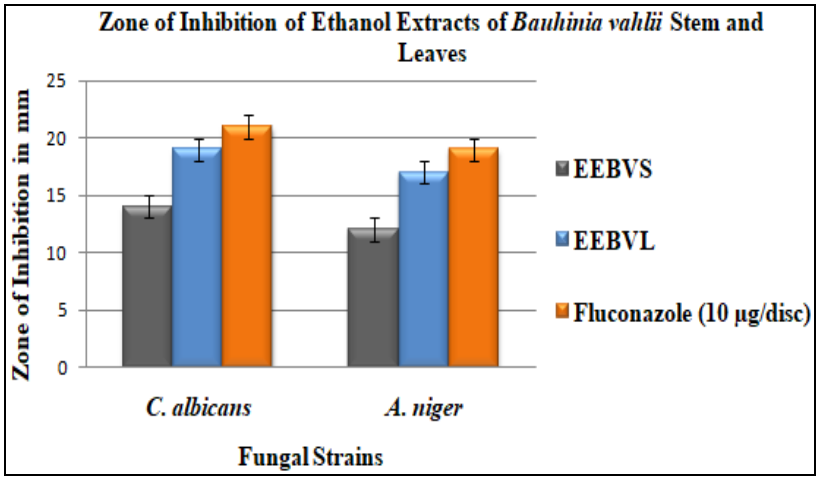

FIG. 4: ZONE OF INHIBITION EEBVS, EEBVL AND STANDARD DRUG ON DIFFERENT FUNGAL STRAINS

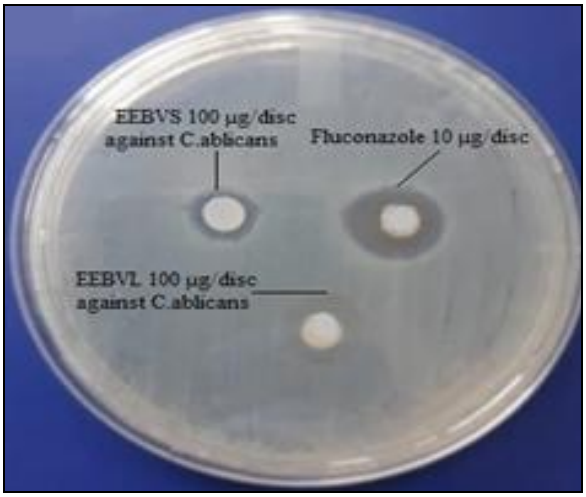

FIG. 5: ANTIFUNGAL ACTIVITY OF EEBVS, EEBVL AND STANDARD DRUG IN THE *CANDIDA ALBICANS*

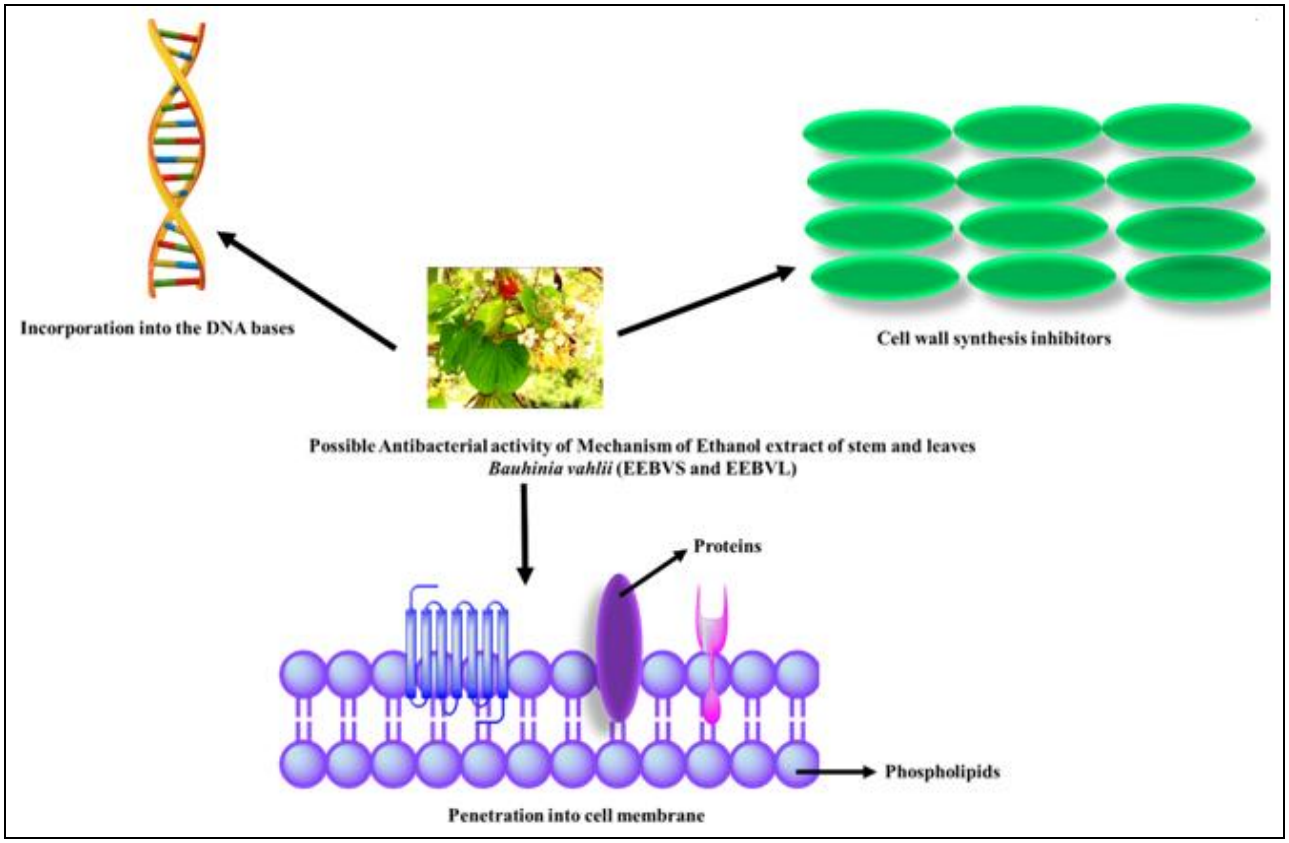

FIG. 6: POSSIBLE MECHANISM OF ANTIBACTERIAL ACTIVITY OF EEBVS, EEBVL

CONCLUSION: In the antibacterial and antifungal activities, both ethanol extracts of leaves and stem showed significant activity against various gram-positive and gram-negative bacteria and fungi strains, whereas a more potent antifungal effect showed in extracts of leaves.

On the other hand, the EEBVL was found to have a more potent effect in the fungal strain against bacterial strain, as observed by zones of inhibitions (ZOI).

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CONFLICT OF INTERESTS: The authors declare no conflict of interest.

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