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Antimicrobial Activity Of Medicinal Plant *Bauhinia variegata Linn.*

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Research Article

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ABSTRACT

The present study attempts to evaluate the antimicrobial efficacy of the ethanol extract of Bauhinia variegata leaves (EBV). Antibacterial activity was tested against both gram positive and gram negative bacteria. Antifungal activity was evaluated against two dermatophytes (Trychophyton mentagrophytes ,Trychophyton rubrum), one non- dermatophyte (Aspergillus niger)and against two plant pathogen (Fusarium Oxysporum and Mucor hiemalis). EBV showed varying degree of inhibitory potential against all the tested bacteria and fungi. EBV showed maximum inhibitory activity against Salmonella typhi (27mm). This is followed by Vibrio cholera, K.Pneumonia, E.coli and S.aureus. The least activity was observed for S.aureus(18mm). For fungi, the EBV was very effective against dermatophyte,T.mentagrophytes and against the plant pathogen, Mucor hiemalis. The antimicrobial activities of EBV may be due to their phytochemical content. Further studies are carried out to isolate and characterize the bioactive compounds responsible for this antimicrobial activity .

KEYWORDS: *Bauhinia variegata, antibacterial, antifungal, phytochemicals, medicinal plant*

Introduction

Antibiotic resistance has become a global concern in recent years. This problem is of great significance especially in developing countries because infectious diseases are one of the major causes of mortality in these countries. The screening of natural products has been the source of innumerable therapeutic agents [1]. Higher plants, as a source for new potential drugs is still largely unexplored and only a small percentage of them has been subjected to phytochemical investigation and the fractions submitted to pharmacological screening is very low. Such screening of various natural organic compounds and identifying active agents is the need of the hour as due to successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay of later in drug development.

Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases [2]. The plant extracts have been developed and proposed for use as antimicrobial substances [3]. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine[4].Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential[5,6,7]. Due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs [8]. Antimicrobial activities of many plants have been reported by the researchers[9,10]. The antimicrobial activities of medicinal plants can be attributed to the secondary metabolites

such as alkaloids, flavonoids, tannins, terpenoids that are present in these plants [11].

Bauhinia variegata linn. (Mandharai) is a medium sized, deciduous tree, found throughout India, ascending to an attitude of 1,300 m in the Himalayas. It is commonly known as Kanchnar in Sanskrit and mountain Ebony in English. In Sanskrit the Kanchnar means "A glowing beautiful lady". The various parts of the plant viz., flower buds, flowers, stem, stem bark, leaves, seeds and roots are practiced in various indigenous systems of medicine and popular among the various ethnic groups in India for the cure of variety of aliments [12,13] .

Various researchers have reported that *Bauhinia variegata* has antidiabetic activity [14,15], good insecticidal [16,17], antigoiterogenic [18] and better antioxidant [19] activity. Bodakhe SH and R.Alpana (2007) [20] have reported that EBV has hepatoprotective property. It is also reported as an anti-inflammatory [21] and immunomodulatory [22] for various inflammatory diseases. *Bauhinia variegata* also has antihyperlipidemic [23] activity. The saline extract of *B.variegata* seed exhibited haemagglutination activity against erythrocytes of man, monkey, rabbit, goat, rat, buffalo, sheep, cow, horse, mule and fowl [24].

The aim of the present study was to evaluate the antimicrobial potential of *B.variegata* leaves so that this medicinal plant could serve as a good candidate to treat various infectious diseases.

MATERIALS AND METHODS:

B.variegata leaves were collected from Chennai and it was authenticated by Dr. P.Jayaraman, Director, National Institute of Herbal Science (authentication reference no. PARC/2010/670 dated 22/12/2010).

Sample preparation

The leaves were washed with water, shade dried and powdered coarsely. Crude extract was obtained after maceration with 95% ethanol at room temperature for 72 hrs, and repeated till exhaustion of the material. Thereafter, the ethanol crude extract was distilled, evaporated and dried under reduced pressure to yield ethanol extract of *B.variegata leaves,*EBV (yield 8%).

Microorganisms

Microorganisms were obtained from stock cultures of department of Microbiology,CSMDRIA and from SRM medical College hospital. Both gram positive and gram negative bacteria were used as test organism for this study. Gram positive *Staphylococcus aureus* ATCC 6538 and gram negative organisms like *Escherichia coli ATCC 9837,Klebsiella pneumonia ATCC 13883,Salmonella typhi ATCC 43579, Vibrio cholera ATCC 14033* were used for antibacterial assay.

Fungal Species like *Aspergillus niger* ATCC 10335, *Trychophyton mentagrophytes* ATCC 28185, *Trychophyton rubrum* ATCC 28188, *Fusarium Oxysporum ATCC 10960, Candida albicans* ATCC 10231. *and Mucor hiemalis ATCC 8977a* were used for antifungal assay.

The organisms were sub cultured on to nutrient agar in order to determine their viability. The identity of each test organism was confirmed by using standard culture, morphological and biochemical techniques as described [25]. Stock cultures were maintained on nutrient agar slants at 4^{O}C and then sub-cultured in nutrient broth at 37^{O}C prior to each antimicrobial test. Inoculants of the test organisms were standardized by methods [26]. This was done by suspending 5 colonies of a 24 hrs culture in 5 ml of nutrient broth and comparing the turbidity with that of Macfarina standards after incubating at 35^{o}C for 2 hrs.

Then the plant extract fractions were subjected to antimicrobial assay using disc diffusion method [27,28,29,30] .

Antibacterial Assay

Muller Hinton Agar was prepared according to the manufacturer's instructions. The medium was sterilized by autoclaving at 121 ^{o}C for 15 minutes at 15 psi pressure and was used for tests. Sterile molten cool (45 ^{o}C) agar was poured aseptically into sterile petridishes (15 ml each) and the plates were allowed to solidify at room temperature in sterile condition. After solidification and drying, the plates were seeded with appropriate micro organisms by streaking evenly on to the surface of the medium with a sterile cotton swab or pouring the appropriate microorganism on the surface of dry agar plate present in peptone broth. Care was taken for the even distribution of culture all over the plate. The inoculums were allowed to dry for 5 minutes. The discs of 6 mm diameter were prepared from Whatmann filter paper No. 1 and were sterilized. The discs were then impregnated with the extracts, solvent DMSO and streptomycin (5 µg/disc) was used as standard. Sterile Whatmann No 1 filter paper with different test concentrations ranging from 100 -1000 µg/disc were placed on to the agar with flamed forceps and gently pressed down to ensure contact along with the diluted extract, one appropriate control dry disc also placed at the center. Then the plates were incubated below 37^{o}C for 24 hrs to allow perfusion of drugs being tested. The next day the zones of inhibition were measured with a measuring scale. This experiment was carried out in triplicate for their confirmation. The results were read by the presence or absence of zone of inhibition.

Antifungal assay

Antifungal activity of an extract was determined by antifungal susceptibility test. The potato dextrose agar (Hi Media) was prepared according to the manufacturer's instructions. The medium was sterilized by autoclaving at 121 ^{o}C for 15 minutes at 15 psi pressure and was used for the assay. After sterilization, 1000µl of EBV was added to all the media containing conical flasks except control flask. The positive control was 20 µl of Amphotericin B (100mg/ml). The media was poured on to the sterile petriplates, and then the plates were allowed to solidify. After solidification 72 hrs fungal cultures were inoculated as 5µl on to the petriplates and kept at 28^{o}C. The % of inhibition was determined for three days. The diameters of the largest and smallest fungal colonies were recorded and the averages were calculated. The inhibition ratios were calculated with the following formula.

Inhibition ratio (%) = (C-E)/Cx100

Where,

C = The average diameter of largest and smallest colonies of the control groups.

E = The average diameter of largest and smallest colonies of the experimental groups.

RESULTS AND DISCUSSION

Antibacterial Analysis of EBV

Table 1 shows the antibacterial effect of EBV. The Result obtained in the present study revealed that EBV possess potential antibacterial activity against all the five tested bacterial organisms (*E.coli, S.aureus, K.pneumonia, S. typhi and V.cholera).*

Table 1: Antibacterial Effect of EBV.

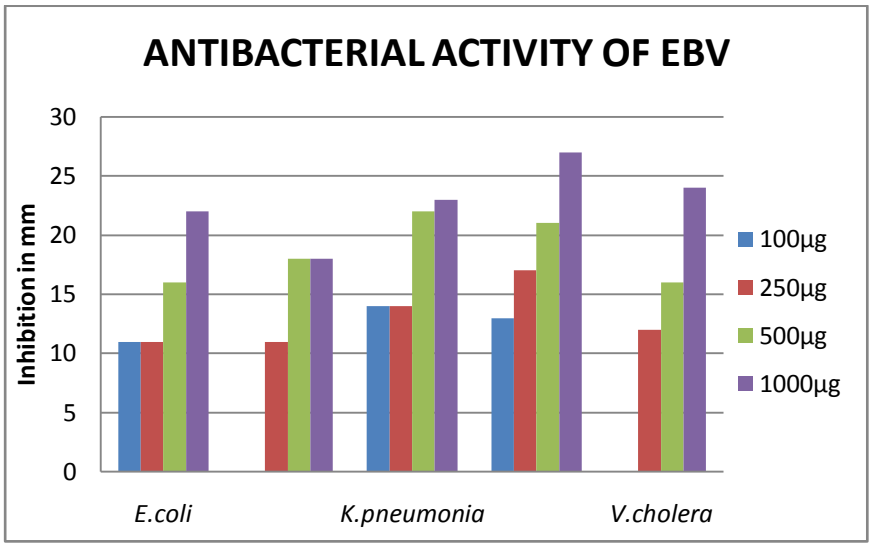
S.No.	Organism	Zone of Inhibition(mm)			
		100µg	250µg	500µg	1000µg
1.	*Escherichia coli*	11	11	16	22
2.	*Staphylococcus aureus*	-	11	18	18
3.	*Klebsiella pneumonia*	14	14	22	23
4.	*Salmonella typhi*	13	17	21	27
5.	*Vibrio cholera*	-	12	16	24

The EBV showed a broad spectrum of activity against all the bacterial strains at the tested concentration of 100 - 1000 µg/disc. Except *S.aureus* and *V.cholera,* all the three remaining organisms were inhibited at *100* µg/disc concentration. EBV showed antibacterial activity against *S.aureus* and *V.cholera* at a concentration of 250- 1000 µg/disc (Graph 1 & Table 1). EBV exhibited greater zone of inhibition for *Salmonella typhi*(27mm). This is followed by *Vibrio cholera, K.Pneumonia, E.coli and S.aureus.* The least activity was observed for *S.aureus*(18mm) at 1000 µg/disc. The antimicrobial activity have been screened because of their great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, becomes an ever increasing therapeutic problem [31,32]. The presence of antifungal and antimicrobial substances in the higher plants is well established as they have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine have been used for the treatment of diseases as done in cases of Unani and Ayurvedic system of medicines, a natural blueprint for the development of new drugs. Much of the exploration and utilization of natural product as antimicrobial arise from microbial sources.

Plant based antimicrobial represents the vast untapped source for medicine. Plant based antimicrobials have enormous therapeutic potential as they can solve the purpose without any side effects that are often associated with synthetic antimicrobials, continued further research and exploration of plant derived antimicrobials is needed today. Medicinal plants were important source for the development of potential, new chemotherapeutic drugs and the *in vitro* antibacterial test form the basis [33,34]. Many of the studies were useful in identifying the active principle responsible for such potentials and to develop clinically important therapeutic drugs for mankind. Hence an attempt has been made to identify the antibacterial activity of leaf extract (ethanol) of *Bauhinia variegata.* Many plants have limitless ability to synthesize

secondary metabolites of which at least 12000 have been isolated. These substances serve as plant defense mechanism against predation by microorganisms, insects and herbivores [35]. Many plants and their extracts used against microbial infections due to the presence of secondary metabolites such as phenols [36]; essential oils [37,38] terpenoids [39,40]; alkaloids [41] and flavanoids [42]. *Bauhinia variegata* leaves were a potential source of phenols, tannins, flavonoids, steroids and of cardiac glycosides. The antibacterial activity of EBV might reside in their phytochemical content. Thus it could be a good candidate for antibiotic formulation.

Graph 1: Antibacterial Effect of EBV.



ANTIFUNGAL ASSAY:

Table 3 illustrates the antifungal property of EBV. 100 µg/ml of EBV was used for this study. The percentage of growth inhibition was determined after 48 hrs and 72 hrs. Amphotericin B was used as the positive control.

From the result, it was evident that EBV has good antifungal activity after 48 hrs. For *T.mentagrophytes* and *Mucor hiemalis*., the activity remains increasing even after 72 hrs, where the activity of positive control starts declining after 72 hrs (**Graph 2 & Graph 3**) .

The EBV was very effective against dermatophyte,*T.mentagrophytes* and against the plant pathogen, *Mucor hiemalis*. Sharma *et al*.,1996 [43] has reported that methanolic leaf extract of *Bauhinia variegata* shows antifungal activity against *Aspergillus fumigates ant Aspergillus niger.*

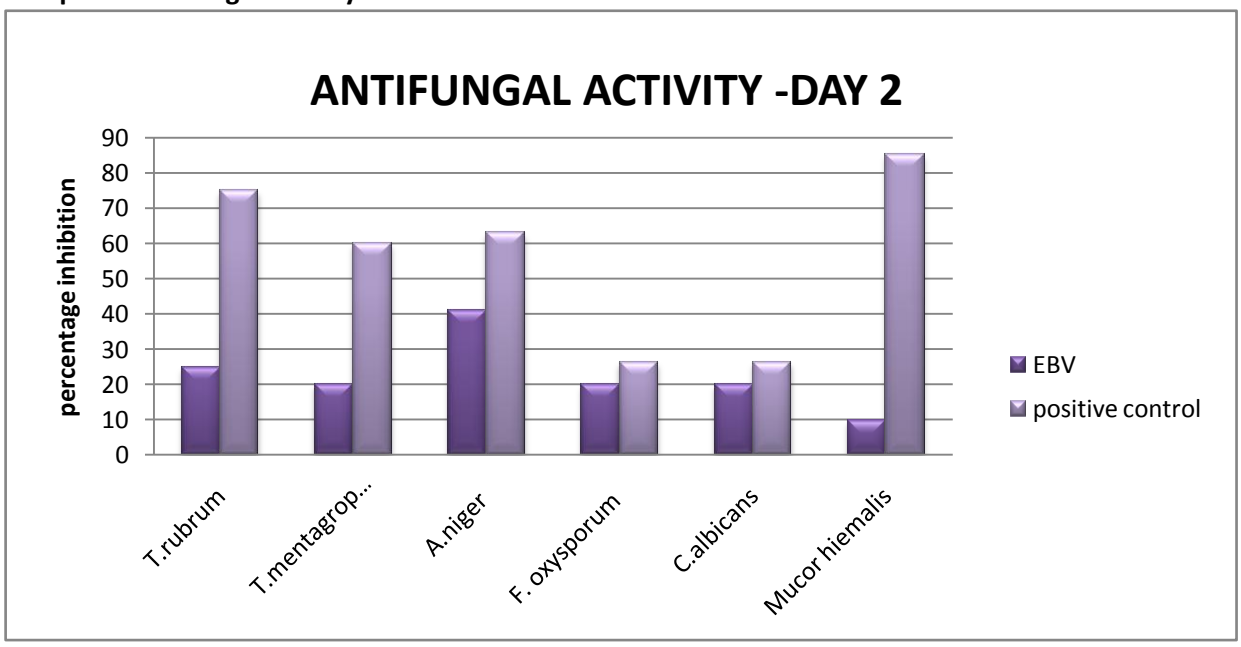
In the present scenario, an emergence of multiple drug resistance in human pathogenic fungi and the small number of antifungal classes available stimulated research directed towards the discovery of novel antifungal agents from other sources, such as medicinal plants [44]. There is very little information available on the activity of medicinal plants.

Table 3. Antifungal Activity of EBV

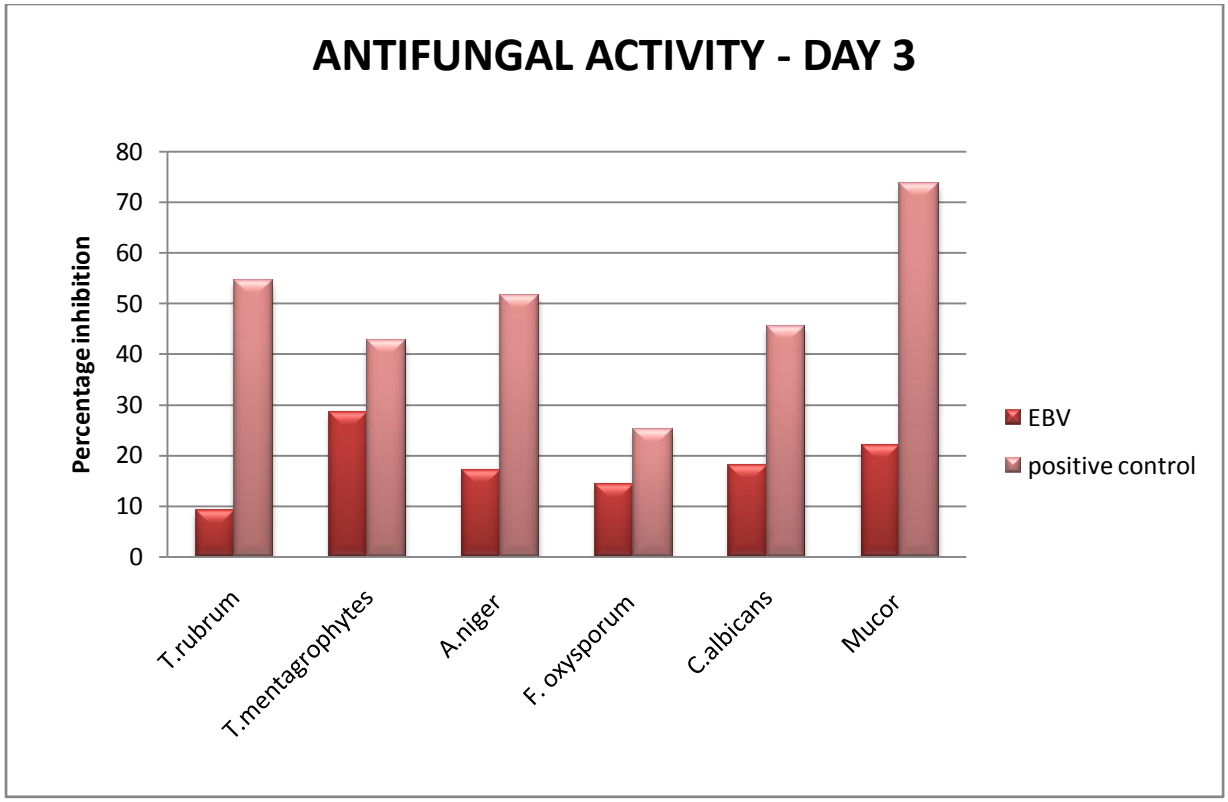
Organism	II day		III day	
	EBV (µg)	'Positive' Control (µg)	EBV (µg)	'Positive' Control (µg)
T.rubrum	25%	75%	9.09%	54.5%
T.mentagrophytes	20%	60%	28.5%	42.8%
A.niger	40.90%	63%	17.2%	51.7%
F. oxysporum	20%	26.31%	14.2%	25%
C.albicans	20%	26.31%	18.1%	45.4%
Mucor hiemalis	9.83%	85.24%	22.1%	73.6%

Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth (antimicrobial activity) [45,46]. The substances that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs. The present study revealed that EBV was effective against various pathogenic fungi. The inhibition after 48 hrs was greater to *A.niger* followed by *F.oxysporum, T.rubrum* and *T. mentagrophytes.* Though, *M.hiemalis* was having least percent of inhibition after 48 hrs. The inhibition percentage increases when incubated for 72 hrs. Hence EBV could be a potential souce of new antibiotics against pathogenic fungi.

Graph 2 : Antifungal activity of EBV after 48 hrs.



Graph 3 : Antifungal activity of EBV after 72 hrs.



CONCLUSION

The plant extract studied could be an answer to the people seeking for better therapeutic agents from natural sources which is believed to be more efficient with little or no side effects when compared to the commonly used synthetic chemotherapeutic agents. The present study verified the traditional use of *B.variegata* for various human ailments especially for various infectious diseases. Thus this plant could be utilized as an alternative source of useful antimicrobial drugs. Further studies are needed to isolate, characterize and elucidate the structure of the bioactive compounds of this plant for antimicrobial drug formulation.

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