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ANTIMICROBIAL ACTIVITY OF STEM BARK OF *BAUHINIA VARIEGATA* LINN.

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ABSTRACT

The present study aimed at evaluating the *in vitro* antimicrobial activity of various extracts of the stem bark of *Bauhinia variegata* Linn. In the current study, the petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extract of stem bark of *B. variegata L.* was tested against standard bacterial and fungal cultures. The test was performed by agar well diffusion method on nutrient agar media and Sabouraud's dextrose media for bacterial and fungal cultures respectively. Among the solvent extracts, ethanolic extract was effective against all the tested bacteria and fungi. Ethanolic extract exhibited maximum inhibitory activity against *Klebsiella.* And *the* least activity was observed for *Staphylococus aureus.* For fungi ethanolic extract was more effective against Aspergillus *fumigates.*

Key words: Antibacterial, Antifungal, *Bauhinia variegata* L.

INTRODUCTION:

Infectious diseases are one of the major causes of health hazard in humans and animals. These infections are caused by pathogenic bacteria, virus and fungi. Severity of infectious disease is bases on virulence factors produced by infectious agent. In recent years, various human pathogens have been reported to acquire resistance toward the common drugs. Drug resistant

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microbes are highly lethal and they increase the severity of infection especially in immuno-compromised patients. Antimicrobial research always looks for new potent antimicrobial drugs from alternative sources. And the Medicinal plants proved to be a major source of new drug discovery. Many plants and plant derivatives are used to cure severe diseases in traditional medicinal system[1].

Bauhinia vareigata L. is a species of flowering plant in the family Caesalpiniaceae. Found wild in the sub-Himalayan tract and outer Himalaya upto 1300m; in Punjab, dry deciduous forests, especially on rocky hills throughout India[2]. The various parts of the plant viz., flower buds, flowers, stem bark, leaves, seeds and roots are practiced in various indigenous systems and popular among various ethnic groups in India for the cure of variety of ailments. The bark is astringent, tonic and anthelmintic. It is also used for ulcers and leprosy. A decoction of the bark is taken for dysentery. It is used to give tone and vitality to body. It is used against tuberculosis and skin ailments. This plant is used in malaria and also an antidote to snake poison[3, 4]. Following a large number of claims on wide range of folk curative properties of *B. vareigata* L. considerable efforts have been made by the researchers to justify its efficacy as a curative agent through pharmacological evaluation.

In this current study we focused on the antimicrobial activity of various extracts of stem bark of *B. vareigata* L. against clinical isolates of Gram positive bacteria, Gram negative bacteria and fungi.

MATERIALS AND METHODS:

Collection and authentification of the plant:

The stem bark of *Bauhinia variegata* L. was collected from Chitradurga, Karnataka. It was authenticated by Prof. V.T. Hiremath, Department of Botany, SJM College of Arts, Commerce & Science, Chitradurga, Karnataka. A voucher specimen no 108-A is deposited in the herbarium of S J M College of Pharmacy, Chitradurga.

Preparation of Crude Extracts:

The stem bark of *Bauhinia variegata* L. was dried in shade and pulverized. The powder is then subjected to soxhlet extraction for continuous hot extraction with petroleum ether, chloroform, ethyl acetate, ethanol (95%) and distilled water. Then each extracts were filtered and filtrate was concentrated under vacuum using rotary vacuum evaporator. The extracts obtained are subjected for screening of antimicrobial activity.

Test microorganisms:

Four pathogenic bacteria, viz., *Staphylococcus aureus*(MTCC737), *Escherichia coli* (MTCC1687), *Streptococcus fecalis* (MTCC3086), *Klebsiella pneumoniae* (ATCC700721), and two pathogenic fungi, viz., *Candida albicans* and *Aspergillus fumigatus* were collected from Maratha Mandal's College of Pharmacy, Belgaum. The cultures were sub-cultured and maintained on nutrient agar media (bacteria) and Sabouraud's dextrose media (fungi) and stored in refrigerator at 4^0C.

Preliminary Phytochemical screening:

Qualitative analysis was carried out to identify the major natural chemical groups such as alkaloids, steroids, triterpenoids, saponins, tannins and flavonoids[5,6].

Determination of Antimicrobial Activity:

Antibacterial activity[7]:

The activity was performed as Basavaraj H.S, et.al with slight alteration, by cup plate method (diffusion technique). The fresh culture of bacteria are obtained by inoculating bacteria into peptone water liquid media and incubated at 37˚ ± 2˚ C for 18 – 24 hours. This culture mixed with nutrient agar media (20%) and poured into petridishes by following aseptic techniques. After solidification of the media five bores are made at equal distance by using sterile steel cork borer (8 mm diameter). Into these cups different concentrations of standard drugs and plant extracts are introduced. Dimethyl formamide was used as a control. After introduction of standard drugs and all the extracts, the plates were placed in a refrigerator at 8˚ - 10˚ C for proper diffusion of drugs into the media. After two hours of cold incubation, the petriplates are transferred to incubator and maintained at 37˚ ± 2˚ C for 18 – 24 hours. After the incubation period, the petri plates were observed for zone of inhibition by using vernier scale. The results evaluated by comparing the zone of inhibition shown by the extracts with standard drugs. The results are the mean value of zone of inhibition measured in millimeter of two sets. The procaine penicillin and streptomycin were used as standard drugs.

Antifungal Activity[8]:

The activity was performed as S. Siva Sakthi et.al with slight alteration. Fungal inoculums was prepared by inoculating a loop-full of test organisms in 5 ml of Sabouraud's dextrose and incubated at room temperature for 3 days. Determination of antifungal activity by Agar well Diffusion Method .Muller Hinton agar plates were inoculated with test organisms by spreading the fungal inoculum on the surface of the media. Wells (8 mm in diameter) were punched in the

agar. All extracts with different concentrations (0.5mg/ml, 1mg/ml) and standard drug were mixed with 1 ml of Dimethyl sulfoxide (DMSO) and added into the well. Well containing DMSO alone act as a negative control. The plates were incubated at room temperature for 3 days. The antifungal activity was assessed by measuring the diameter of the zone of inhibition (in mm). Ciclopirox olamine was used as standard drug.

RESULTS AND DISCUSSION

Preliminary phytochemical screening:

Reactions in this analysis revealed the presence or absence of phytoconstituents in the crude extracts tested**.** Results are given in Table-1.

Table 1 Preliminary Phyto-chemical screening

Phytoconstituents	Petroleum ether Extract	Chloroform Extract	Ethyl acetate extract	Ethanol Extract	Aqueous extract
Alkaloids	-ve	-ve	-ve	+ve	+ve
Carbohydrates	-ve	-ve	+ve	+ve	-ve
Steroids	+ve	+ve	-ve	+ve	-ve
Triterpenoids	+ve	+ve	-ve	+ve	-ve
Flavonoids	-ve	-ve	+ve	+ve	-ve
Tannins	+ve	+ve	+ve	+ve	+ve
Saponins	+ve	-ve	+ve	+ve	+ve

Antibacterial and Antifungal activity:

Drug resistant in microbes become a big problem along with the emergence of new infectious diseases. Microorganism acquired resistant against the pharmaceutical drugs by the production of drug degrading enzymes, resistant plasmids, alteration of metabolic pathway etc[9].

Table 2 and 3 shows the antibacterial effect of *Bauhinia variegata* L. Analysis of the data revealed that all the five extracts from *Bauhinia variegata* L. showed different ranges of antimicrobial activities. Ethanol fraction has more antibacterial activity against all the tested bacteria among which *Klebsiella pneumoniae (16mm)* was more susceptible bacterium and the resistant strain was *Escherichia coli (12mm).* Petroleum-ether extract exhibited least antimicrobial activity.

Table 4. Shows the antifungal activity of *Bauhinia variegata* L. All the extracts of *Bauhinia variegate* L. showed considerable antifungal activity at both the concentrations (0.5mg/ml and 1 mg/ml) with certain variations. Ethanolic extract is more effective against both the fungi in comparison to other fractions. Ethyl acetate fraction showed more anti-*Klebsiella pneumoniae* activity (18mm) and minimum activity was exhibited by petroleum ether extract.

Aromatic and medicinal plants are known to produce certain bioactive molecules like Tannins, steroids, flavonoids, triterpenoids, and saponins which react with other organisms in the environment, inhibiting bacterial or fungal growth exhibiting antimicrobial activity[10].

Table 2: Antibacterial activity

Sl. No	Name of the compounds	Mean zone of inhibition (in mm)*			
		Staphylococcus aureus		***Escherichia coli***	
		0.5mg/ml	**1mg/ml**	**0.5mg/ml**	**1mg/ml**
01	Procaine penicillin	18	20	-	-
02	Streptomycin	-	-	19	21
03	ALC	10(0.55)	12(0.60)	11(0.57)	13(0.61)
04	PE	9(0.5)	10(0.5)	9(0.47)	12(0.57)
05	AQ	11(0.61)	14(0.7)	8(0.42)	11(0.52)
06	EA	9(0.5)	11(0.55)	8(0.42)	10(0.47)
07	CHL	10(0.55)	12(0.60)	10(0.52)	13(0.61)

Table 3: Antibacterial activity

Sl. No	Name of the compounds	Mean zone of inhibition (in mm)*			
		Streptococcus fecalis		***Klebsiella***	
		0.5mg/ml	**1mg/ml**	**0.5mg/ml**	**1mg/ml**
01	Procaine penicillin	17	19	-	-
02	Streptomycin	-	-	20	21
03	ALC	12(0.70)	14(0.73)	14(0.7)	16(0.76)
04	PE	8(0.47)	11(0.58)	12(0.8)	13(0.85)
05	AQ	9(0.53)	11(0.58)	15(0.75)	16(0.76)
06	EA	10(0.58)	13(0.68)	16(0.8)	16(0.76)
07	CHL	9(0.53)	12(0.61)	15(0.75)	17(0.8)

Table 4: Antifungal activity

Sl. No	Name of the compounds	Mean zone of inhibition (*in mm*)*			
		Candida albicans		***Aspergillus fumigatus***	
		0.5mg/ml	**1mg/ml**	**0.5mg/ml**	**1mg/ml**
01	Ciclopirox olamine	21	23	19	21
02	ALC	11(0.52)	14(0.61)	12(0.63)	14(0.66)
03	PE	12(0.57)	13(0.560	10(0.53)	11(0.52)
04	AQ	12(0.57)	14(0.61)	12(0.63)	13(0.62)
05	EA	11(0.52)	15(0.65)	14(0.73)	16(0.76)
06	CHL	12(0.57)	17(0.73)	11(0.58)	13(0.62)

CONCLUSION:

The present research concluded that the extracts of stem bark of *Bauhinia variegate* L. were endowed with significant antibacterial and antifungal properties, thereby justifying its use in indigenous system of medicine. 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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