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Original Article

A study on proximate analysis and antimicrobial properties of *Bombax ceiba pentandra* fruit and spike extracts

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ABSTRACT

Bombax ceiba pentandra belongs to the family Malavaceae, is being largest exploited for its wide therapeutic applications in various tribal communities around the world. Present study investigated the antibacterial study of five solvent extracts of spike and young fruits using aqueous, methanol, ethyl acetate, chloroform, and Hexane against five bacterial species namely *Escherichia coli, Bacillus Subtilis, Staphyolococcus aureus, Pseudomonas aerogenosa and Shigella flexnerri.* Also Antimycotic study was performed using the five solvent extracts against the fungal cultures namely *Candida albicans, Fusarium oxysporum, Cladosporium bantia, Alternaria brassicae, Curvalaria lunata*. Among all the solvent extracts, methanol extract of fruit and spike exhibited significant Antibacterial and Antimycotic activity. Presence of higher concentration of Polyphenolic and flavanoid compounds in the methanol extract may be responsible for the effective antimicrobial property.

Key words: *Bombax ceiba pentandra*, antibacterial, antimycotic, polyphenols, flavanoids.

INTRODUCTION

The herbal medicines serve the health needs of about 80% of the world's population. More than 65% of the global population uses medicinal plants as a primary health care modality (1). Antimicrobial agents are undeniably one of the most important therapeutic discoveries of the 20th century. Antimicrobial molecules either kill or prevent the growth of microbes. Antibiotics are the most important weapons in fighting microbial infections benefitting the human health. However, with the 'antibiotic era' barely five decades old, mankind is now faced with the global problem of emerging resistance in virtually all pathogens (2). In general, microbes have the genetic ability to transmit and acquire resistance to the therapeutic drugs (3).

In recent years, many possible sources of natural antibiotics are in use and therefore there is a need to screen and develop new drugs either synthetic or natural for the efficient growth inhibition of the microbes. Plants are used medicinally as a source of many potent and powerful drugs (4). The secondary metabolites of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action (5). Considering the vast potentiality of plants as the sources for antimicrobial activities, present study aims for the screening of antibacterial and antimycotic activities for the different solvent extracts of B*ombax ceiba pentandra.*

B. ceiba pentandra is a tropical tree of the order malvales and belongs to the family Malvaceae. This tree is being largely exploited for its wide therapeutic applications in various tribal communities around the world (6). Young fruits of the trees are used in the treatment of snake bite as well as in inflammatory diseases (7). Stem bark decoctions are used in mouth washes for treating toothache and mouth problems, and taken to treat stomach problems, diarrhea, hernia, gonorrhea, heart trouble, edema, fever, asthma, and rickets. They are also applied on swollen fingers, wounds, sores, furuncles and leprous macules (8). Toxicological studies proved that B*ombax ceiba pentandra* has very low toxicity profile in all the tested animals and it is safe for oral medication (9).

Present study investigated the *In-vitro* antibacterial activity of five extracts of spike and young fruits of *Bombax ceiba pentandra* using aqueous, methanol,

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ethyl acetate, chloroform, and Hexane against five bacterial species *Escherichia coli, Bacillus Subtilis, Staphyolococcus aureus, Pseudomonas aerogenosa and Shigella flexnerri*. Also *In-vitro* Antimycotic study was performed against the fungal cultures namely of *Candida albicans, Fusarium oxysporum, Cladosporium bantia, Alternaria brassicae, Curvalaria lunata*. .

MATERIAL AND METHODS

Plants material: Young Fruits, leaves and Spikes of Bombax *ceiba pentandra* were collected from JSS college botanical garden as well as St. Philomena's college campus, Mysore, Karnataka, India and authenticated from the Department of Botany, University Of Mysore, Mysore, Karnataka. Samples were washed then shade dried, powdered, sieved and stored in air tight containers.

Preparation of plant extracts: 100 gm each of the sample was extracted separately with different solvents namely hexane, chloroform, ethyl acetate, methanol and water. The crude residues were obtained by evaporating the solvents in rotary flash evaporator. Each of the residue was reconstituted with respective solvent and stored for further use.

Proximate analysis of the solvents extracts: The proximate composition of different solvent extracts were carried out to determine the content of ascorbic acid, tannins, saponins, glycosides, proteins, total phenols, flavanoids as well as alkaloids. The protein content was estimated by Bradford method (10). Total phenolic contents were estimated using Folin-Ciocalteu reagent (11). Flavanoids were estimated following the method of Woisky and Salatino (12). Ascorbic acid content was estimated using DNPH reagent (13). Qualitative analysis of tannins, alkaloids, saponins, and glycosides were performed for the different extracts (14).

Determination of Antibacterial activity

Preparation of media: 2gm of nutrient broth along with 2gm of agar were dissolved in 100ml distilled water under aseptic condition

Pure culture: Bacterial cultures were obtained from the Department of Studies in Microbiology, University of Mysore, Mysore. These cultures were inoculated to nutrient broth medium and incubated for 16hrs at 37ºc.

Bacterial species and antibiotics: *Escherichia coli, Bacillus subtilis, Pseudomonas aerogenosa, Staphylococcus aureus, Shigella flexnerri.* Ampicillin (1ppm) was used as antibiotic.

***In-vitro* inhibition study:** The *in-vitro* antibacterial activity was carried out against 24hr old culture bacteria. The antibacterial study was conducted for the determination of Zone of Inhibition and MIC (minimum inhibitory concentration). Different concentration of solvents extracts of sample were tested for anti-bacterial activity by well diffusion method. The prepared nutrient agar was poured into petri dish aseptically.

After solidification, Nutrient agar medium was inoculated with different bacteria, six wells are made at equal distance by using sterile micro tips (8mm diameter). The wells were then filled with four different concentrations of solvent extracts namely 50µl, 100µl, 150µl and 200µl against the organisms. Respective solvents were used as control. Agar plates containing bacteria and samples were incubated at 37ºC for 24 hrs. Ampicillin was used as standard antibiotic for the comparison of zone of inhibition against the solvent extracts.

Antimycotic study

Preparation of media: 2gm of PDB as well as 2gm of agar were dissolved in 100ml distilled water under aseptic condition.

Pure culture: Pure Fungal cultures were obtained from Department of Microbiology, St. Philoemeona's College, Mysore. The cultures were inoculated to PDB broth and incubated for 7days for 25ºc.

Fungal species and antibiotic: *Candida albicans, Fusarium oxyporum, Cladosporium bantia, Alternaria brassicae, Curvalaria lunata.* Flucanazole (1ppm) was used as standard antibiotic for the comparision of zone of inhibition against the solvents extract

***In-vitro* inhibition study:** The in-vitro antifungal activity was carried out against 72 hours old culture of fungi. The antifungal study was conducted for the determination of following parameters like, Zone of Inhibition and MIC (minimum inhibitory concentration). Different concentration of solvents extracts were tested for anti-fungal activity by well diffusion method. The prepared potato dextrose agar was poured into petri dish aseptically.

After solidification, potato dextrose agar medium was inoculated with fungi, six wells are made at equal distance by using sterile micro tips (8mm diameter). The wells were then filled with four different concentration of solvents concentration namely 50µl, 100µl, 150µl and 200µl in against fungal organisms. Respective solvents were used as control. Agar plates containing fungi and solvents

extracts were incubated at room temperature (27°c) for 48 hrs.

RESULTS

Proximate analysis: Table 1, Shows the results of proximate analysis of *B. ceiba pentandra* spike and fruit extracts. Considerable amount of protein and ascorbic acid were reported in the aqueous extract of the fruits as compared to that of spikes. Higher levels of flavanoids and polyphenols were present in the methanol extract of fruits and spikes in comparison to hexane, ethyl acetate and aqueous extracts.

Antibacterial activity: Table 2 refers to the antibacterial activity. Among the five solvent extracts of B. ceiba pentandra fruit extracts tested against *Escherichia coli, Bacillus subtilis, Pseudomonas aerogenosa, Staphylococcus aureus, Shigella flexnerri,* methanolic extract of fruit exhibited significant growth inhibition against all the test bacterial species. A maximum zone of inhibition of 16mm was observed for methanolic extract of fruits against *E.coli* and *B. subtilis* at a concentration of 200µg/ml. Fruit methanolic extract at a concentration of 200µg/ml inhibited the growth of bacterium *S.aureus* and *S.flexnerri* with the zone of inhibition of about 15mm and 14mm for the bacterium *P.aerogenosa*.

A dose dependent growth inhibition was monitored against all the 5 bacterial species studied. The antibacterial potency of the methanolic extract of fruits was notable in comparison to the antibiotic Ampicillin (1ppm) used. Ethyl acetate extract of fruits revealed growth inhibition against only *E.coli* bacterium.

Methanol extract of *B. ceiba pentandra* spike exhibited considerable growth inhibition of 15 mm and 16mm against E.coli and B.subtilis respectively at a concentration of 200µg/ml. A zone of inhibition of 16mm was observed for ethyl acetate extract of spike against *E.coli* species.

Antimycotic activity: Tables 3 refers to the antimycotic activity. Out of the five solvent extracts of B. ceiba pentandra fruits, only methanolic extract showed significant antimycotic activity. A maximum zone of inhibition of 18mm and 17mm was observed against the fungal species C.albicans and *Curvalaria lunata* respectively. Against *Alternaria brassicae, Cladosporium bantia and Fusarium oxyporum* species methanol extract exhibited growth inhibition of 15mm, 14mm and 12mm respectively. The fungal growth inhibition by methanolic extract of fruits was much significant in comparison to the standard antibiotic Flucanazole used against the test organisms.

DISCUSSION

In recent years the use of herbs in traditional medicine has gained attention as they are being proven as the promising sources of various bioactive molecules with novel mechanism of action (15). Polyphenols are ubiquitously distributed group of plant secondary metabolites ranging from simple molecules like phenolic acids, phenyl propanoids, flavanoids to highly polymerized compounds namely lignins, melanins and tannins. Flavanoids are the most common and widely distributed subgroups. These phytochemicals exhibit diverse physiological and pharmcologoical effects including antiviral, antibacterial, antifungal, anti-inflammatory, hepatoprotective and anticarcinogenic actions (16). Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings.

The present study is an attempt for the preliminary screening of phytochemical components and antimicrobial activities of different solvents extracts of *bombax ceiba pentandra*. Among the five solvent extracts used, methanol extract of fruits exhibited significant growth inhibition for all the bacteria and fungi species studied. Antimicrobial activity was observed against few pathogenic microorganisms including the gram-ve bacterial species. The zone of inhibition was significantly more in comparison to the standard antibiotic i,e the methanol extract of fruit could effectively inhibited the growth of bacteria and fungi.

The proximal composition analysis showed higher concentration of total phenols, flavanoids as well as tannins in the methanol extracts of fruits and spikes. The effective antibacterial and antimycotic activities of methanol extract of fruit could be endorsed for the presence of higher concentration of phenolic and flavanoid compounds. Earlier studies have reported the role of polyphenols and tannins in the growth inhibition of *Escherichia coli, Staphylococcus aureus, salmonella typhi and Candida albicans*. Flavanoids are classified under phenolic groups in plants which have been known to possess antimicrobial activity. The antimicrobial action of flavanoids can be due to the inhibition of nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism (17).

Phenolic compounds reporting the antimicrobial property perhaps have the mechanism of action

involving the alteration of the permeability of the cell membrane that could result in the uncoupling of oxidative phosphorylation, inhibition of active transport, and loss of pool metabolites due to cytoplasmic membrane damage. Also, the presence of hydroxyl group in phenolic compound might influence their antimicrobial effectiveness by binding to the active site of enzymes, form hydrogen bonds with enzymes and alter their metabolism, and also the lipid solubility and the degree of stearic hindrance of the phenolic compounds might determine their antimicrobial activity (18). Many phytochemicals exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites (19).

CONCLUSION

The antimicrobial effect of the methanolic extract of *Bombax ceiba pentandra* fruits could be endorsed for the presence of wide spectrum of bioactive molecules including polyphenols, flavanoids and tannins. Further studies could introduce the plant as a potential candidate for drug development for the treatment of ailments caused by pathogenic, drug resistant microorganisms.

Fig- 1 Antibacterial activity of methanol extract of *B. ceiba pentandra* fruits

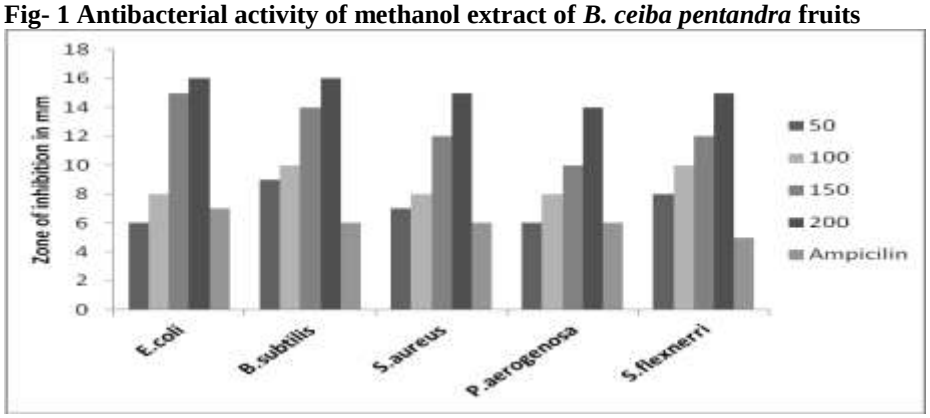

Fig- 2 Antibacterial activity of ethyl acetate extract of *B.ceiba pentandra* fruits

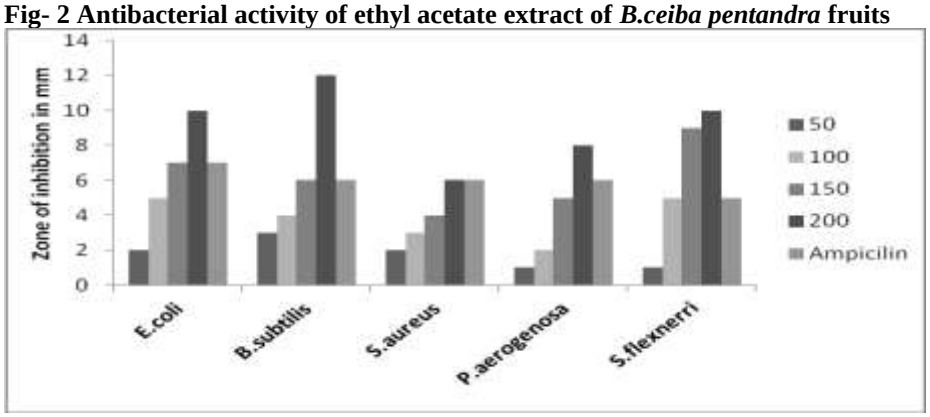

Fig- 3 Antibacterial activity of methanol extract of *B.ceiba pentandra* spikes

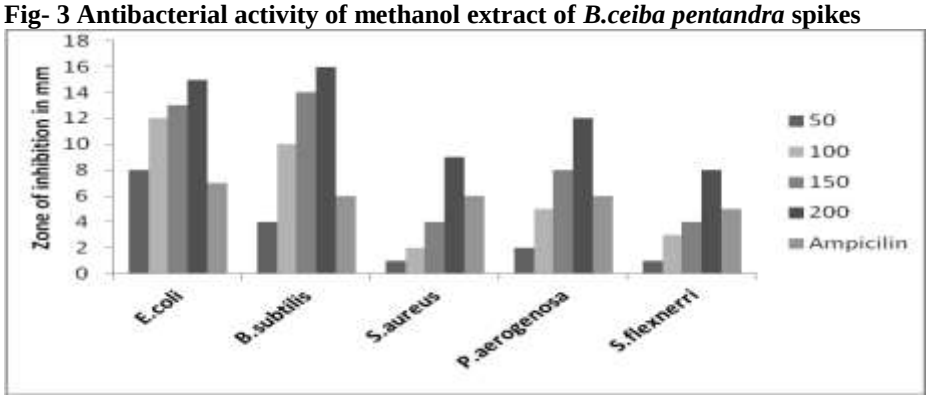

Fig- 4 Antibacterial activity of ethyl acetate extract of *B.ceiba pentandra* spikes

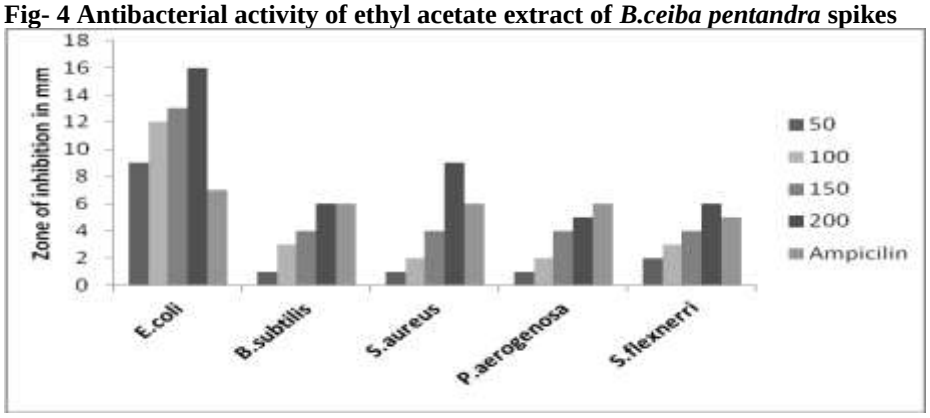

Fig- 5 Antimycotic activity of methanol extract of *B.ceiba pentandra* fruits

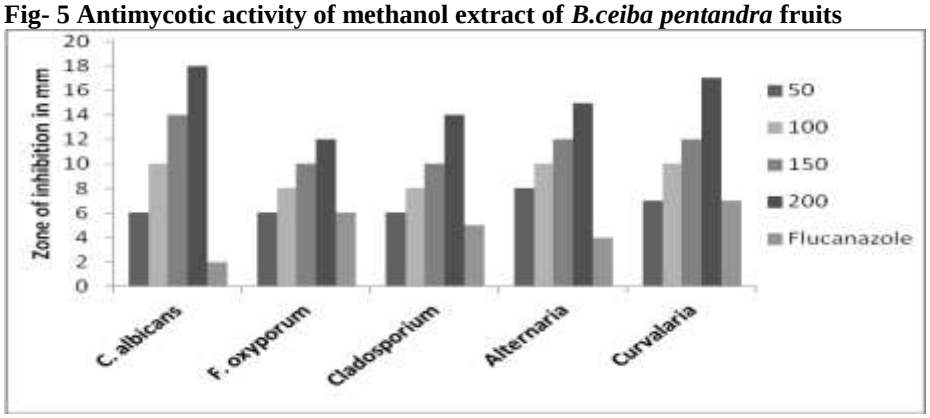

Table 1: Proximate analysis of different solvent extracts of *B. ceiba pentandra* fruit and spike
-: absent, +: low concentration, ++: Moderate concentration, +++: High concentration

Solvent extract	Proteins	Phenols	Flavanoids	Tannins	Alkaloids	Saponins	Ascorbic acid	Glycosides
Aqueous extract(spike)	++	++	++	+	+	+	+	+
Aqueous extract(fruit)	++	++	++	+	+	+	+	+
Methanol extract(spike)	-	+++	+++	++	-	-	-	-
Methanol extract(fruit)	-	+++	+++	+++	-	-	-	-
Ethylacetate extract(spike)	-	+	+	-	-	-	-	-
Ethylacetate extract(Fruit)	-	+	+	-	-	-	-	-
Chloroform extract(spike)	-	+	-	-	-	-	-	-
Chloroform extract(fruit)	-	+	-	-	-	-	-	-
Hexane extract(spike)	-	-	-	-	-	-	-	-
Hexane extract(fruit)	-	-	-	-	-	-	-	-

Table- 2 Antibacterial activity of *Bombax ceiba pentandra* fruit extract

Zone of inhibition in mm									
	Methanol extract (Concentration in µg)				Ethyl acetate extract (Concentration in µg)				Ampicillin (1ppm)
Organism	**50**	**100**	**150**	**200**	**50**	**100**	**150**	**200**	**200**
E.coli	6 ± 0.58	8 ± 0.88	15 ± 0.34	16 ± 0.89	2 ± 1.57	5 ± 0.67	7 ± 0.51	10 ± 0.68	7 ± 0.82
B.subtilis	9 ± 0.51	10 ± 0.16	14 ± 0.91	16 ± 0.60	3 ± 0.58	4 ± 0.89	6 ± 0.98	12 ± 0.89	6 ± 0.98
S.aureus	7 ± 0.07	8 ± 1.25	12 ± 0.76	15 ± 0.48	2 ± 0.06	3 ± 0.47	4 ± 0.90	6 ± 1.08	6 ± 0.57
P.aerogenosa	6 ± 0.08	8 ± 0.92	10 ± 0.83	14 ± 0.91	1 ± 0.09	2 ± 0.77	5 ± 0.67	8 ± 0.78	**6** ± 0.67
S.flexnerri	8 ± 0.29	10 ±0.15	12 ± 0.28	15 ±0.67	1 ± 0.07	5 ± 0.29	9 ± 0.84	10 ± 0.69	5 ± 0.09

Values are means ± SEM; n = 3

Table - 3 Antibacterial activity of *Bombax ceiba pentandra* spike extract

Zone of inhibition in mm									
	Methanol extract (Concentration in µg)				Ethyl acetate extract (Concentration in µg)				Ampicillin (1ppm)
Organism	**50**	**100**	**150**	**200**	**50**	**100**	**150**	**200**	**200**
E.coli	8 ± 0.66	12 ± 0.51	13 ±1.01	15 ± 1.09	9 ± 0.57	12 ± 0.33	13 ± 0.88	16 ± 0.03	7 ± 0.57
B.subtilis	4 ± 0.33	10 ± 0.88	14 ± 0.16	16 ± 0.33	1 ± 0.88	3 ± 0.16	4 ± 0.33	6 ± 0.19	6 ± 0.88
S.aureus	1 ± 0.57	2 ± 0.66	4 ± 0.69	9 ± 0.34	1 ± 0.75	2 ± 0.66	4 ± 0.69	9 ± 0.34	6 ± 0.57
P.aerogenosa	2 ± 0.66	5 ± 0.33	8 ± 0.03	12 ± 0.57	1 ± 0.60	2 ± 0.30	4 ± 0.03	5 ± 0.66	6 ± 0.67
S.flexnerri	1± 0.57	3 ± 1.20	4 ± 0.35	8 ± 0.03	2 ± 0.33	3 ± 1.02	4 ± 0.33	6 ± 0.83	5 ± 0.09

Values are means ± SEM; n = 3

Table- 4 Antimycotic activity of *Bombax ceiba pentandra* fruit extract

	Methanol extract (Concentration in µg)				Flucanazole (1ppm)
Organism	**50**	**100**	**150**	**200**	**200**
C. albicans	6 ± 0.68	10 ± 1.25	14 ± 0.98	18 ± 0.57	2 ± 0.66
F. oxyporum	6 ± 0.07	8 ± 0.70	10 ± 0.82	12 ± 0.67	6 ± 0.89
Cladosporium	6 ± 0.91	8 ± 0.82	10 ± 0.60	14 ± 0.33	5 ± 1.20
Alternaria	8 ± 0.82	10 ± 0.60	12 ± 0.83	15 ± 0.88	4 ± 0.89
Curvalaria	7 ± 0.16	10 ± 0.48	12 ± 0.34	17 ± 0.51	7 ± 0.57

Values are means ± SEM; n = 3

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