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Antimicrobial activity of *Butea frondosa* Roxb

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Abstract- *Butea frondosa* plant was used in traditional medicine for the treatment of night blindness, dysentery, piles, ulcers, tumors, menustrual disorders, cough, stromatitis, leprocy in early stages, skin diseases etc. Based on the traditional Knowledge about the plant, present study was conducted. To evaluate the scientific basis for the use of plant, the antimicrobial activities of the extracts at different doses (10mg and 20mg/ml) of the leaves were evaluated against some common pathogenic bacteria and fungi using agar disc diffusion method. Gram positive bacteria like staphylococcus aureus, gram negative bacteria like *Pseudomonas aeruginosa, Klebsiella Pneumoniae, Escherichia coli, Proteus mirabilis, Proteus vulgaris* and fungi like *Aspergillus niger, Aspergillus flavus* were used and antimicrobial activity of the concentrated extracts was evaluated by the diameter zone of inhibition against the above microorganisms. Plant extracts were active against both gram positive, gram negative Bacteria and fungi. The above observation indicates that, *Butea frondosa* has broad spectrum antibacterial and antifungal activity and a potential source of new classes of antibiotics that could be useful for infectious disease chemotherapy and control. The study also conducted on the isolation of the chemical constituents present in the plant. The Phytochemical constituents of the dried powdered plant leaves were extracted using aqueous and organic solvents, Results revealed the presence of carbohydrates, Glycosides, Alkaloids ,steroids, Tannins, Phenols.
Keywords: Antimicrobial activity, *Butea frondosa*, Infectious diseases, Medicinal plants

INTRODUCTION

Plant remains the most common source of antimicrobial agents. Their usage as traditional health remedies is the most popular for 80% of world population in Asia, Latin America and Africa; it is reported to have minimal side effects [1, 2]. In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products, extracted from plants to produce more cost effective remedies that are affordable to the population. The rising incidence in multidrug resistance amongst pathogenic microbes has further necessisated the need to search for newer antibiotic sources [3]. *Butea frondosa* Roxb is a small tree which grows to a height of 12 to 15 meters. In the summer months, when most of the trees and shrubs are dry due to the scorching heat of the sun, *Butea frondosa* synonemous to *Butea monosperma* truly stands out like a flame in the forest with its orange coloured flowers very often it has a crooked trunk and irregular branches [4, 5]. The trifoliate oval leaves are more prominent in all other seasons except the summer months when it is time for floral buds to be activated. The leaves of this tree are collected by the local people and a few of them are oven together with small twigs to make circular disposable biodegradable leaf plates to serve food. These plates are then used by small large groups of people during specific occasions like marriages, picnics [6] Because of its wide meditional use and availability. This study was setout to investigate the antimicrobial activity of the plant.

MATERIALS AND METHODS

Plant materials were collected from the nearest fields of Gulbarga university campus in Karnataka State India and were Identified and authenticated by the department of botany in Gulbarga university

Preparation of extracts:

50grams of the powder of *Butea frondosa* leaf and its components were extracted separately with various extracts like Petroleum ether, chloroform, Ethanol, Aqueous(400ml) at respective boiling point of extracts. The was filtered using wattman filter paper and concentrated to dryness under reduced pressure.

Test organisms

Both gram positive and gram negative bacterial isolates and some fungi were used for this work. They include *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus vulgaris, E. coli* and fungi like *Aspergillus niger, Aspergillus flavus* They were obtained from Department of Microbiology, Gulbarga university Gulbarga ,Karnataka. India. All the bacteria were suspended in nutrient broth and fungi in potato dextrose agar medium [16]. Nutrient agar and potato dextrose agar were used for testing antibacterial and antifungal activity with the different extracts of *Butea frondosa*.

Determination of antimicrobial activity:

Antimicrobial activity of organic solvent extracts of the plant was evaluated by agar diffusion method. Freshly prepared 24hr culture were taken and spread on to the sterile nutrient agar plates [13].spreading was done using swab which was spread on to the plates uniformly then wells were punched into the plates using a sterile gel puncher, the wells were maintained as positive control with standard antibiotic like streptomycin and other four wells were with three extracts, petroleum ether, chloroform, ethanol and water dissolved in DMSO (10mg/ml and20mg/ml) and fungal culture *Aspergillus niger, Aspergillus flavus*, were spread on potato dextrose agar medium with extracts of same concentration (10mg/ml and20mg/ml)was used, inoculated bacterial cultures were incubated at 37°c for 24hr and fungal cultures for 48hr [14]. Antibacterial and antifungal activity was determined by measurement of zone of inhibition around each well in plate.

RESULTS AND DISCUSSION

Phytochemical studies revealed the presence of Alkaloids, carbohydrates, steroids, Saponins, Tannins, and Phenols Glycosides etc which is shown in table 1. Alkaloids, carbohydrates, steroids, amino acids and proteins were detected in all the four extracts. Glycosides were found in ethanolic and water extracts, Tannins were found in ethanolic and water extracts. Tannins were present in chloroform, ethanol and water extract. Phenols were found in ethanol and Aqueous extract, But they are absent in petroleum ether and chloroform extract. Phytochemical constituents such as saponins, Alkaloids, glycosides, flavonoids, tannins, steroids several other aromatic compounds are secondary metabolites of plant that serve as difference mechanism against predation by many microorganisms, insects and herbivores. Results of antimicrobial activity of plant extracts were shown in Table 2. Extracts of concentration (10mg/ml&20mg/ml) were used for study .The results shown that plant extracts were effective against both gram positive and gram negative bacteria and with fungi also. The successive extract of Butea frondosa have been investigated for antimicrobial activity against *Staphylococcus aureus , Pseudomonas aeruginosa, Klebsiella pneumoniae, E.coli* and fungi like *Aspergillus niger, Aspergillus flavus,* highest zone of inhibition was shown by Chloroform extract of 20mg/ml concentration with *Staphylococcus aureus* and *Pseudomonas aeruginosa* it was 28mm,Ethanol extract shown highest zone of inhibition with *Klebsiella pneumoniae* it was 28mm.petroleum ether extract inhibited staphylococcus aureus of 10mg and 20mg/ml by 18mm and 20mm,chloroform extract inhibition was 25mm and 28mm,Ethanol extract inhibition was 28mm and 20mm,water extract inhibition was 18mm and 20mm,std zone with streptomycin was 30mm. *Pseudomonas aeruginosa* inhibition with petroleum ether extract of concentration 10mg and 20mg/ml was 20mm and 22mm,chloroform inhibition was 26mm and 28mm,Ethanol extract inhibition was 26mm and 26mm,water extract inhibition was 22mm and 22mm,standard zone was 28mm. *Klebsiella pneumoniae* inhibition with petroleum ether was 20mm and 24mm,chloroform extract inhibition was 28mm and 26mm,Ethanol extract inhibition was 28mm and 24mm,water extract inhibition was 20mm and 22mm,standard zone was 18mm.*Escherichia coli* inhibition with petroleum ether extract was 20mm and 22mm,chloroform extract inhibition was 20mm and 20mm,Ethanol extract inhibition was 22mm and 22mm,water extract inhibition was 22mm and 20mm.standard zone was 20mm.proteus mirabilis inhibition with petroleum ether extract was 20mm and 14mm,chloroform extract inhibition was 18mm and 20mm,Ethanol extract inhibition was 18mm and 20mm,water extract inhibition was 20mm and 20mm,standard zone was 25mm.*Proteus vulgaris* inhibition with petroleum ether extract was 14mm and 16mm,chloroform extract inhibition was 20mm and 18mm,Ethanol extract inhibition was 20mm and 16mm,water extract inhibition was 14mm and 12mm,Standard zone was 20mm.*Aspergillus niger* inhibition with petroleum ether extract was

21mm and 20mm,chloroform extract inhibition was 23mm and 18mm.Ethanol extract inhibition was 21mm and 20mm.water extract inhibition was 13mm and 20mm.standard zone was 18mm.*Aspergillus flavus* inhibition with petroleum ether extract was 15mm and 09mm,Chloroform extract inhibition was 15mm and 11mm.Ethanol extract inhibition was 12mm and 11mm,water extract inhibition was 14mm and 16mm,standard zone was 15mm.All the four extracts have shown antimicrobial activity when compared with the std drug streptomycin(10mg/ml).

In 1999 the antimicrobial activity of *Ocimum americanum.L.* Essential oil by some bacteria and fungi for evaluations of antimicrobial activity, here filter paper disc diffusion method was used. The essential oil showed promising activities against *E.coli, B.megaterium* and *Staphylococcus aureus*. The maximum activity was exhibited by B.megaterium, also showed the wide range of activity with all the tested fungi [7].In 1997 antimicrobial activity against 24hrs cultures of three selected bacteria using petroleum ether, chloroform and ethanol extracts was done. Bacteria used were *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *fungus Aspergillus niger* was also used. It was performed by cup plate method using gentamycin and nystatin as standards. Inhibition was recorded by measuring the diameter zone of inhibition. Chloroform and ethanolic extracts showed the high antibacterial and antifungal activity against all the tested microorganisms. Petroleum ether revealed the lowest zone of inhibition with Psedomonas aeroginosa [8]. In the study with Butea frondosa it also revealed the antimicrobial property of this plant.

The demonstration of antibacterial and antifungal activity against both gram positive and gram negative bacteria and fungi may be indicative of presence of broad spectrum antibiotic compounds. This will be immense advantage in fighting the menace of antibiotic refractive pathogens that are so prevalent in recent times [15].In 1999 In vitro Antifungal activity was done by using Essential oil from Luvunga scadens,oil showed moderate inhibitory effect [9].Bioactive principles are widely distributed among the higher plants [10].Many surveys include an assessment of how rationally the antibiotics has been prescribed [11].Excessive and appropriate use of antibiotics in Hospitals contributes to the development of bacterial resistance and to increased hospital costs. This is the reason for intensive drug utilization evaluations [12]. Demonstration of broad spectrum of antibacterial and antifungal activity by Butea frondosa may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control.

Finally all the Bacteria and fungi showed the zone of inhibition with all the extracts.

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Table 1- Phytochemical Screening Of Butea Fronosa Leaves

SN	Test	Petroleum etherextract	Chloroform extract	Ethanol extract	Water extract
1	Carbohydrate: molisch Test , Anthrone Test	+ —	+ +	— +	+ —
2	Protein & Aminoacids :Ninhydrin Test	+ +	+ +	+ +	+ +
3	Steroids: Salkowskiis Test Liebermann Burchards Test	+ +	+ +	+ +	+ +
4	Phenolic compound: Elagic acid Test phenol Test	— —	— —	+ +	+ +
5	Glycosides: Kellar killani Test, Sulphuric acid Test	— —	— —	+ +	+ +
6	Saponins: FoamTest	+	+	+	+
7	Tannins: FeCl3 Test Gelatin Test	— —	+ +	+ +	+ +
8	Alkaloids: Mayers Test Dragendorffs Test Wagners Test	+ + +	+ + +	+ + +	+ + +
9	Flavonoids: Lead acetate Test	— —	— —	— —	— —

Table 2- Showing Antimicrobial Activities of Butea Frondosa

	Microorganisms	Zone of inhibition in mm									
		Petroleum ether		chloroform		Ethanol		water		Streptomycin	Flucanozole
		10 Mg/ml	20 Mg/ml	10 Mg/ml	20 Mg/ml	10 Mg/ml	20 Mg/ml	10 Mg/ml	20 Mg/ml	10 Mg/ml	10 Mg/ml
1	*S.aureus*	18	20	25	28	28	20	18	20	30	NT
2	*P. aeruginosa*	20	22	26	28	26	26	22	22	28	NT
3	*K.Pneumoniae*	20	24	28	26	28	24	20	22	18	NT
4	*Escherichia coli*	20	22	20	20	22	22	22	20	20	NT
5	*Proteus mirabilis*	20	14	18	20	18	20	20	20	25	NT
6	*Proteus vulgaris*	14	16	20	18	20	16	14	12	20	NT
7	*Aspergillus niger*	21	20	23	18	21	20	13	20	NT	18
8	*Aspergillus flavous*	15	09	15	11	12	11	14	16	NT	15

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