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Research Article

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EVALUATION OF PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY OF *BUTEA MONOSPERMA* LEAF EXTRACTS

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ABSTRACT
The antibacterial activity of ethanol and aqueous extracts of leaves of *Butea monosperma* was evaluated on one gram positive strain like *Staphylococcus aureus* and one gram negative strain like *Escherichia coli*. The *invitro* antibacterial activity was performed by disc diffusion method. The disc diffusion method for antibiotic susceptibility testing is the Kirby-Bauer method. The significant antibacterial activity of the active extracts was compared with standard antibiotic gentamicin (40µg/ml).From the experiment done the ethanolic extract of *Butea monosperma* leaves did produce considerable antibacterial activity than the aqueous extract was observed. In addition the preliminary phytochemical tests of ethanolic and aqueous extract of *Butea monosperma* leaves revealed the presence of the alkaloids, carbohydrates, tannins, flavanoids, phenolic compounds and starch. The results obtained in the present study suggest that *Butea monosperma* leaves can be used in treating diseases caused by test organisms.
KEY WORDS: *Butea monosperma*, ethanolic extract, aqueous extract, Multi -drug resistant bacteria, Phytochemicals.

INTRODUCTION

India is a land of biodiversity. The plants are the source of medicines since ancient times, According to world health organization, 80% of the populations in the world depend on traditional medical practitioners for their medicinal needs. Many plant formulations and their products are being used in treatment of diseases. Resistance to antibiotics is becoming a difficult problem hence there is a need to develop new approaches in the treatment of diseases.

Butea monosperma[1] (Lam) is a deciduous tree, belongs to family Fabaceae, which grows up to 15 m in height and 1.5- 1.8 m in girth, with a crooked trunk. Bark light- brown or bluish grey, yielding a ruby-red vitreous gum. Wood white or yellowish brown, often becoming grey or grayish- brown. Leaves 3- foliolate, large, unequal, 10.2-20.4 cm. Flowers borne in racemes, brilliant orange red, 3.8-5.1 cm long. Lower calyx-teeth deltoid. Pods silvery-white, broad dehiscing by one suture. Seeds flat, elliptic, reddish-grey, 3.2cm6.

Butea monosperma Lam is a wild crop and grows in most parts of India as a tree. It is reputed in systems of medicines as the various parts of the plant *Butea monosperma*[1,2] has been used traditionally for many of the diseases like anti-inflammatory, antimicrobial, anthelmintic, antidiabetic, diuretic, analgesic, antitumor, anticancer, astringent etc. The leaves are useful in diabetes[3], in treatment of leucorrhea and also useful in congested and septic throat. Leaves are given in diarrohea heartburn, diabetes, flatulence, piles and worms. The leaves and seeds are useful as, in hemorrhage, astringent, diuretic and have anti-implantation and antiovulatory properties. Flowers have aphrodisiac and tonic properties. Bark are used in tumors, bleeding piles, ulcers and have inhibitory action against E.coli and Micrococcus pyrogens. Roots are used to cure night blindness. Chemical component of *Butea monosperma* are alkaloids and recently reported Euphane triterpenoid ester and pterocarpan. Seed contains palasonin,-d-methyl cantharidin, infections caused by ·-amyrin, sitosterol and alkaloid- monospermine. Glycerides of palmitic, stearic, linoceric, oleic and linoleic acids, proteolytic and lipolytic enzymes. While bark contains tannins and gum (Butea gum), leucocyanidin and its tetramer procyanidin, gallic acid and mucilaginous material. Its flowers contain isobutin, coreoopsin, monospermoside and their isoderivatives.

MATERIALS & METHODS

Plant Material

Leaves of *Butea monosperma* plant was collected from local region of Narsapur, District of Medak, Andhra Pradesh, India in the month of Jun 2010. The botanical identity was confirmed by a botanist Prof T.Mohana Department of Botany, Government Mehbubia Junior College, Gunfoundry, Hyderabad. (Reference No: 3/2010), Gentamicin which used as a standard in this experiment was purchased from local market (manufactured by Concord Drugs Limited, batch number 91215) All reagents and chemicals used were AR grade.

Preparation of Extracts

5 Kg of leaves of *Butea monosperma* were shade dried, crushed to coarse powder and passed through sieve # 44. The sieved powder was stored in air tight, high density poly ethylene containers before extraction. Extraction was performed by using soxhlet apparatus (48 hours), carried out with ethanol and aqueous[4] (separately). The extracts were concentrated for further studies at reduced pressure and temperature in a rotary evaporator and tested for presence of secondary metabolites by different phytochemical tests. Different concentrations of extract were prepared by dissolving the fine powder in 10% aqueous Dimethylsulfoxide (DMSO) for further study.

Preparation of bacteria

The bacteria *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Escherichia Coli* were purchased from M.T.C.C Institute of Microbial Technology, Chandigarh, India (Invoice No. 9/7/5790). The ability of the various extracts to inhibit growth of clinical bacteria and fungi isolates was determined using the Agar disc diffusion method[5]. Sterile filter paper discs, 11 mm in diameter were impregnated with each extract concentration and dried at 30° C in the static incubator. They were then carefully placed aseptically with a forceps on the surface of the Mueller-Hinton (MH) agar plates that were pre-inoculated with the 24 hr culture of bacteria and 0.1 ml spore suspension (1 x 10^5 spores/ml). The control antibiotics disc containing gentamicin (40µg/ml) was placed on each of the inoculated plates of nutrient agar. The plates were left on the bench undisturbed for few minutes, after which the bacterial culture plates were incubated at 37° C for 24 h. The external diameters of visible zones of growth inhibition were measured after incubation.

Screening of antibacterial activity

The disc diffusion method was used for the determination of the antibacterial activity.

Disc diffusion method

Screening of extracts for antibacterial activity was done by the disc diffusion method. It was performed using an 18 h culture at 37°C in 10 ml of Mueller Hinton broth. Bacterial inoculums were spread over the plates containing Mueller-Hinton agar[6] using a sterile cotton swab to get a uniform microbial growth on both control and test plates. The extracts were dissolved in 10% aqueous Dimethyl Sulfoxide (DMSO). Under aseptic conditions, empty sterilized discs (What man no.5 mm dia) were impregnated with 100 µl of each of the extracts of different concentration and left to dry under laminar flow cabinet and then placed on the agar surface. Standard discs containing gentamicin (40µg/ml) was used as reference control.

All Petri dishes were left for 30 min at room temperature to allow the diffusion of test drugs and kept for incubation at 37°C for 18 h. After the incubation time, all the plates were examined for the presence of zones of inhibition. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured to the nearest millimeter (mm) as observed.

RESULTS AND DISCUSSION

The phytochemical active compounds of *Butea monosperma* leaves were qualitatively analyzed and the results were presented in Table-1. In the phytochemical screening[7,8] compounds such as alkaloids, carbohydrates, flavanoids, tannins and phenolic compounds present in *Butea monosperma.* Knowing the phytochemical constituent can help one to speculate on the medicinal value of the leaves. Tannins have antimicrobial properties. Alkaloids have pronounced physiological effect. The presence of there phytochemicals in the leaves suggests that the plant is pharmacologically active, supporting the claim by traditional healers. The first step towards this goal is the in *vitro* antibacterial activity assay. In the present study antibacterial activities were analyzed against *Staphylococcus aureus* and *Escherichia coli*. The antibacterial assay[9] was performed using disc diffusion method. The highest antibacterial activities were observed in ethanolic extract (18.07±0.845, 26.87±1.42, 30.83±0.920, 38.1±0.850) than the aqueous extract (16.77±0.800, 24.5±1.95, 28.83±0.33, 35.5±2.88). There is low antibacterial activity in aqueous extract. The results obtained were tabulated and a graphical representation of the antibacterial activity of the extracts was done.

This result indicated that ethanolic extract of *Butea monosperma*[10] can be used for treatment the infectious diseases caused by gram-positive bacteria such as S. aureus and E. coli, which are gram negative bacteria. This result proves that the use of the leaves of *Butea monosperma*[11] to cure several illnesses, especially those caused by microbes, is valid. It is expected that the results to from this study would serve as background knowledge for further studies on the plant, which would result to discovery other medicinally useful properties.

CONCLUSION

Ethanolic extract of *Butea monosperma* possesses antimicrobial potentials against both gram positive and gram negative bacteria. It is therefore confirmed as a useful antimicrobial agent. The powdered leaves is rich in phytochemicals and secondary metabolites such as alkaloids, carbohydrates, flavanoids, tannins and phenolic compounds which are probably responsible for its medicinal properties.

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Table1: Phytochemical screening of *Butea monosperma* leaves extracts

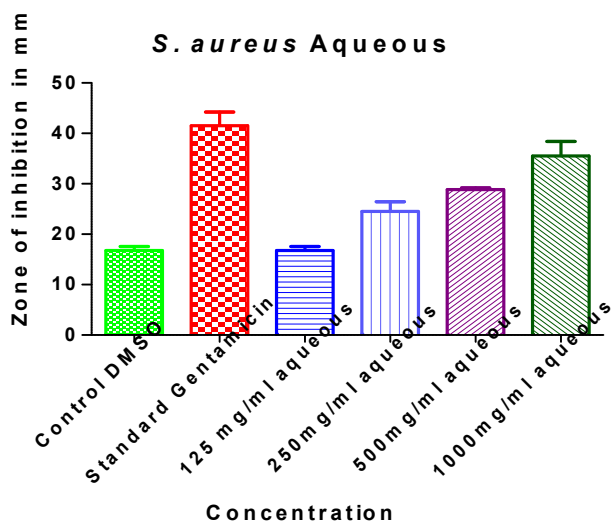
Constituents	Ethanolic Extract	Aqueous Extract
Alkaloids	+	+
Sterols	-	-
Glycosides	-	-
Carbohydrates	+	+
Protiens	-	-
Cardiac Glycosides	-	-
Saponins	-	-
Tannins & Phenolic Compuonds	+	+
Starch	+	+
Flavonoids	+	+

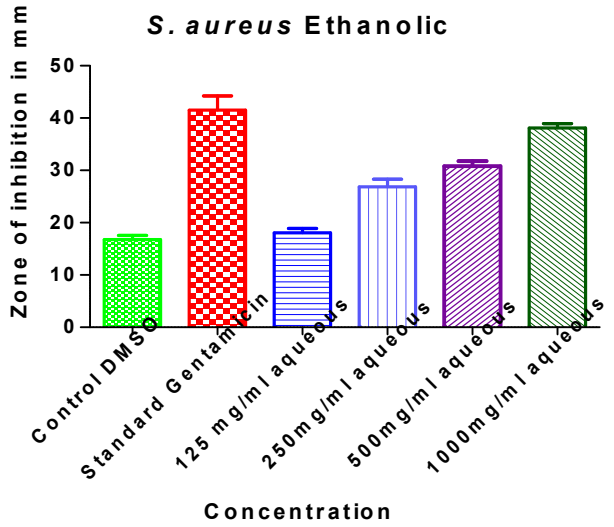
Table: 2: Screening of antibacterial activity of *Butea monosperma* leaves extracts

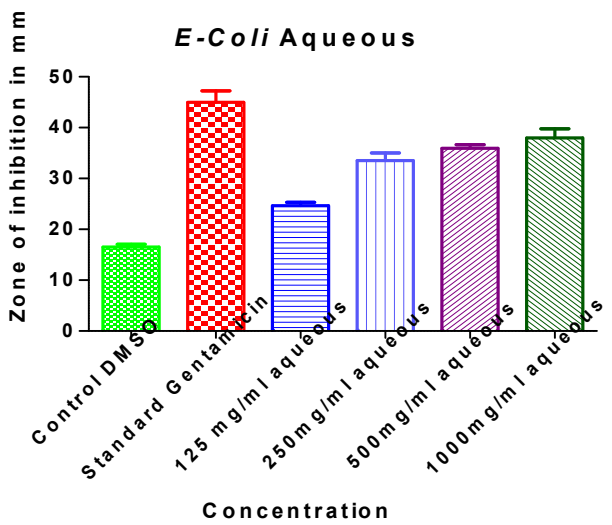
Treatment	*Staphylococcus aureus* (Zone of inhibition in mm)		*Escherichia coli* (Zone of inhibition in mm)	
	Ethanolic Extract	Aqueous Extract	Ethanolic Extract	Aqueous Extract
Control (DMSO)	16.77±0.775	16.77±0.775	16.50±0.577	16.50±0.577
40µg/ml Gentamicin	41.53±2.69***	41.53±2.69***	44.97±2.26***	44.97±2.26***
125 mg/ml	18.07±0.845	16.77±0.800	23.17±2.52*	24.63±0.664**
250 mg/ml	26.87±1.42**	24.5±1.95*	28.63±0.648***	33.5±1.528***
500 mg/ml	30.83±0.920***	28.83±0.33**	32.8±0.929***	35.93±0.721***
1000 mg/ml	38.1±0.850***	35.5±2.88***	38.47±0.636***	37.97±1.810***

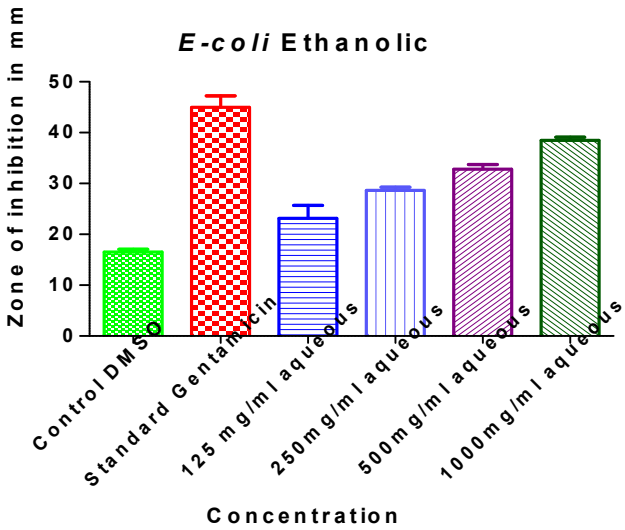
Values are expressed as Mean + SEM. and analyzed by One way Analysis of variance (ANOVA) followed by Dunnet`s t test n=8, *P< 0.05, **P<0.01, ***P<0.001.

Graphical representation









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