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PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIMICROBIAL ACTIVITY OF *BUTEA MONOSPERMA* (L) BARK ETHANOLIC AND AQUEOUS EXTRACT

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ABSTRACT

Infectious diseases are the second leading cause of death worldwide. Treatment of infections continues to be problematic in modern time because of the severe side effects of some drugs and the growing resistance to antimicrobial agents. *Butea monosperma* (L) bark ethanolic and aqueous extract has shown good efficacy against *Bacillus cereus*. *Pseudomonas aerugenosa* and *Escherichia Coli* in concentration dependent manner which were grown on MH- Agar medium. 1000mg/ml has shown better zone of inhibition (ZOI) in all the cases of above micro organisms

INTRODUCTION: The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind [1]. Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug-resistant bacteria. It is essential to investigate newer drugs with lesser resistance [2].

The use of herbs and medicinal plant as the first medicines is a universal phenomenon. Every culture on the earth, through written or oral tradition, has relied on the vast variety of natural chemistries found in plants for their therapeutic properties. All drugs from the plant are substances with a particular therapeutic action extracted from plants [3]. *Butea monosperma* (Lam) is a deciduous tree, belongs to family fabaceae, which grows up to 15 m in height and 1.5- 1.8m in girth, with a crooked trunk. Bark is 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, 10.2-20.4cm. Flowers are borne in racemes, brilliant orange red, 3.8-5.1cm long, lower calyx-teeth deltoid, pods silvery- white, broad dehiscing by one suture. Seeds are flat, elliptic, reddish- grey, 3.2cm [4].

MATERIALS AND METHODS

Plant Material: Bark of *Butea monosperma* plant was collected from local region of Narsapur, District of Medak, Andhra Pradesh, India in the month of June, 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)

Preparation of Extracts: 5 Kg of bark was 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 (12 hours), carried out first with petroleum ether (60-80 ^{o}C) to defat the material. The defatted material was then extracted with ethanol to get ethanolic extract and finally with water and small quantity of Chloroform (as 6.5ml/litre) as preservative to get aqueous extract. The aqueous and ethanolic 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 [5].

Preliminary Phytochemical Analysis: The bark extract was screened for the phytochemical components using the standard Method [5, 6]. The phytochemical components analyzed were alkaloids, steroids, starch, proteins, anthraquinone glycosides, saponins, flavonoids, tannins, and cardiac glycosides.

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. 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 [8] 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 (40 µg/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 ^{o}C for 24 h. The external diameters of visible zones of growth inhibition were measured after incubation [7].

RESULTS: From the **Figure 1** and **2** and **table 1** and **2**, both ethanolic extract and aqueous extract of *Butea monosperma* (L) has shown significant effect at 1000mg/ml as compared with the standard gentamicin. It is more effective against Bacillus aureus and E. coli in Concentration dependent manner. From **table 3** we can confirm that presence of alkaloids, tannins, phenolics, carbohydrates and saponins in both extracts.

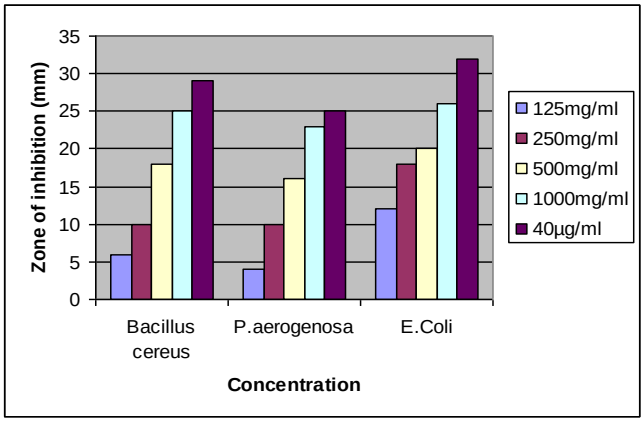


FIGURE 1: INDICATES ZONE OF INHIBITION WITH THE ETHANOLIC EXTRACT

Indicates 125mg/ml of Ethanolic extract
Indicates 250mg/ml of Ethanolic extract
Indicates 500mg/ml of Ethanolic extract
Indicates 1000mg/ml of Ethanolic extract
Indicates 40µg/ml of Gentamicin

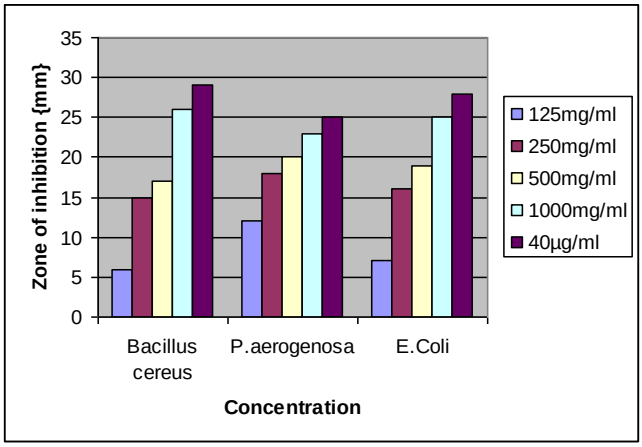


FIGURE 2: INDICATES ZONE OF INHIBITION WITH THE AQUEOUS EXTRACT

Indicates 125mg/ml of aqueous extract
Indicates 250mg/ml of aqueous extract
Indicates 500mg/ml of aqueous extract
Indicates 1000mg/ml of aqueous extract
Indicates 40µg/ml of Gentamicin

TABLE 1: PHYTOCHEMICAL SCREENINGS OF *BUTEA MONOSPERMA* BARK ETHANOLIC EXTRACT

Constituents	Ethanolic extract	Aqueous extract
Alkaloids	+	+
Sterols	-	-
Glycosides	-	-
Carbohydrates	+	+
Mucilage	-	-
Starch		
(a) Iodine test	-	+
(b) Tannic acid test	-	+
Proteins	-	-
Cardiac glycosides	-	-
Saponins	+	+
Tannins and Phenolics	+	+
Flavonoids	-	-

+ indicates presence of active constituents
- indicates absence of active constituents

TABLE 2: DIAMETERS (MM) OF ZONES OF INHIBITIONS PRODUCED BY *BUTEA MONOSPERMA* (L) BARK ETHANOLIC EXTRACT

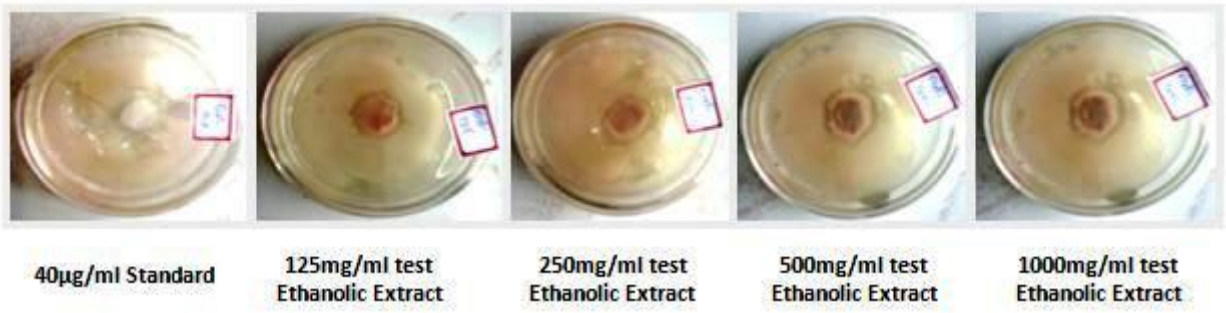
Microorganism Species	Standard 40µg/ml Gentamicin ZOI in mm	125mg/ml *Ethanolic extract* ZOI in mm	250mg/ml *Ethanolic extract* ZOI in mm	500mg/ml *Ethanolic extract* ZOI in mm	1000mg/ml *Ethanolic extract* ZOI in mm
Bacillus cereus	29	6	10	18	25
Pseudomonas aerogenosa	25	4	10	16	23
Escherichia Coli	32	12	18	20	26

TABLE 3: DIAMETERS (MM) OF ZONES OF INHIBITIONS PRODUCED BY *BUTEA MONOSPERMA* (L) BARK AQUEOUS EXTRACT

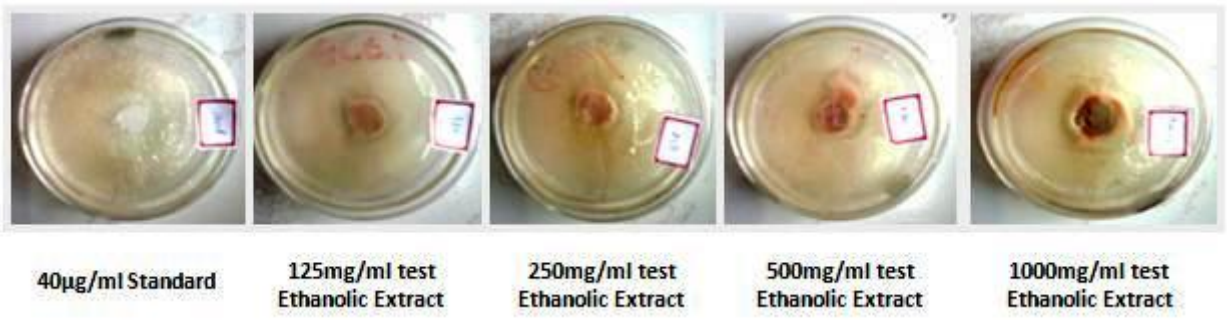
Microorganism Species	Standard 40µg/ml Gentamicin ZOI in mm	125mg/ml *Ethanolic extract* ZOI in mm	250mg/ml *Ethanolic extract* ZOI in mm	500mg/ml *Ethanolic extract* ZOI in mm	1000mg/ml *Ethanolic extract* ZOI in mm
Bacillus cereus	29	6	15	17	26
Pseudomonas aerogenosa	25	12	18	20	23
Escherichia Coli	28	7	16	19	25

Following pictures indicates Microbial Zone of inhibition of cultured *Bacillus cereus* at different concentrations of ethanolic extract of *Butea monosperma* (L).

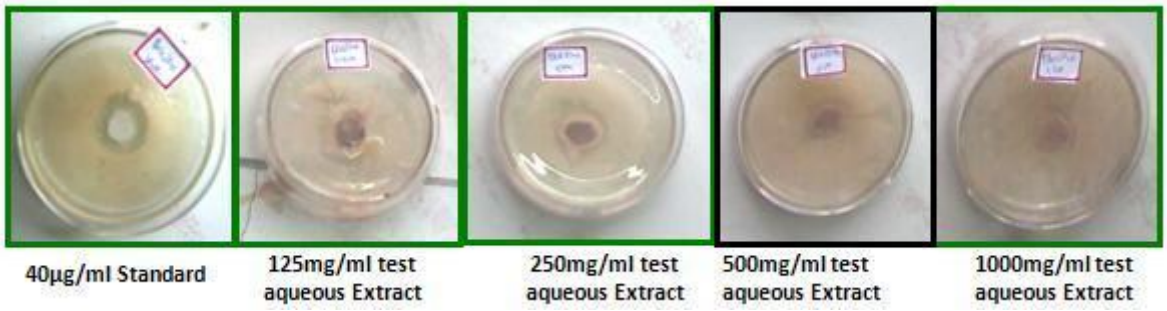
Following pictures indicating Microbial Zone of inhibition of cultured *Escherichia coli* at different concentrations of ethanolic extract of *Butea monosperma* (L).



Following pictures indicating Microbial Zone of inhibition of cultured *Pseudomonas aerugenosa* at different concentrations of ethanolic extract of *Butea monosperma* (L).

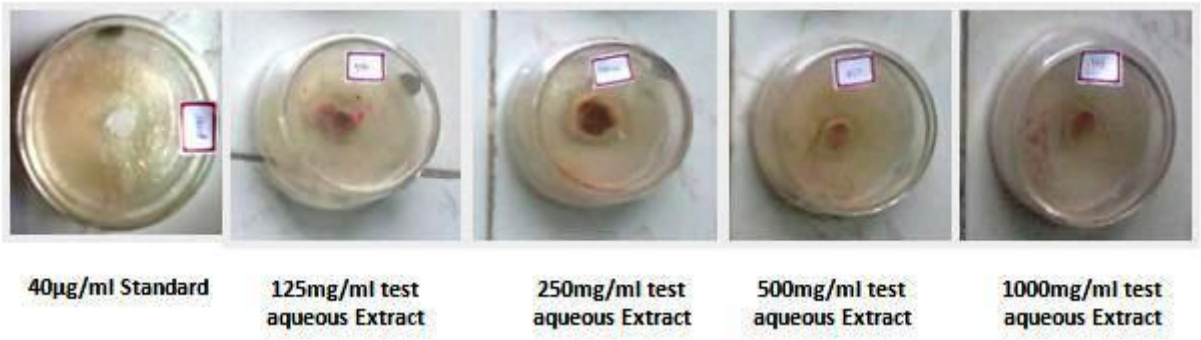


Following pictures indicates Microbial Zone of inhibition of cultured *Bacillus cereus* at different concentrations of aqueous extract of *Butea monosperma* (L).



Following pictures indicates Microbial Zone of inhibition of cultured *E. coli* at different concentrations of aqueous extract of *Butea monosperma* (L).

Following pictures indicates Microbial Zone of inhibition of cultured *Pseudomonas aerugenosa* at different concentrations of aqueous extract of *Butea monosperma* (L).



DISCUSSION: Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay. Many reports are available on the antiviral, antibacterial, antifungal, antihelmintic, antimolluscal and anti-inflammatory properties of plants. 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 [1]. However, not many reports are available on the tannins have been traditionally used for protection of Inflamed surfaces of the mouth and treatment of catarrha, wounds, haemorrhoids, and diarrhea, and as antidote in heavy metal poisoning. Flavonoids are naturally occurring phenols which possess numerous biological activities including anti-inflammatory,

antiallergic, antithrombotic and vasoprotective effects. Cyanogenetic glycosides are reported to possess antimicrobial activity. The observed antimicrobial activity against the tested organisms could be due to the presence of tannins and cyanogenetic glycosides in the extract as these have previously been reported to possess antimicrobial activities. These could explain the rationale for the use of the plant in the treatment of the various conditions in traditional medical practice [9].

CONCLUSION: In conclusion ethanolic and aqueous extract of *Butea monosperma* (L) bark was assessed in this study. The results seem to justify their continued use in the treatment of microbial infections. The inhibition of growth of the test organisms that are known to cause nosocomial infections and displaying multidrug resistance to most antibiotics and non-antibiotic antimicrobial agents justify the continued use of these plants in folk and traditional medical practice. Further investigation on the isolation and identification of anti microbial component(s) in the plant may lead to chemical entities with potential for clinical use.

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