BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, December 2014. Vol. **11**(3), 1695-1698

Investigatory Study on Antimicrobial Activity and Phytochemical Screening of *Butea monosperma* Linn

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doi: http://dx.doi.org/10.13005/bbra/1571

(Received: 16 October 2014; accepted: 25 November 2014)

Infectious diseases are the second leading cause for worldwide death. 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. Over the past few decades the use of antibiotics is under threat as many commonly used antibiotics have become less effective against certain illnesses due to emergence of multi drug-resistant (MDR) bacteria. The continuous search for the source of new antibiotics is needed to face the problem of increasing resistant strains of bacteria. It is known that more than 400,000 species of tropical flowering plants possesses medicinal properties through their phytochemical constituents. Considering the important role of *Butea monosperma* in inhibition of different cultures of bacteria, the present study was conducted to evaluate its antimicrobial spectrum against different life threatening pathogenic microorganisms and screening for various phytochemical constituents.

Kew words: antimicrobial activity, phytochemical screening, MDR bacteria, antimicrobial agents, *Butea monosperma* Linn.

Plants have long been the principal tools of traditional medicinal system. Despite convincing progress in synthetic, traditional and indigenous drugs used by different ethnic groups of the world for treatment of disease have been tested on longtime scale. This is attributed relatively safe nature, easily availability and provided economic to masses. Traditional plants therapies coupled with dietary measures as prescribed in indigenous system of medicines has proven result in a number of health disorders.

India has a high rang of medicinal plants that are used in ancient as well as in modern pharmaceutical preparations, they have used in a preparation of drugs since centuries ago. Due to the potent therapeutic value, easy avability and mode of action these medicinal plants have attended more pharmacological exploration in modern medicinal practices.

The *Butea monosperma* Linn. belongs to the family *Fabaceae* commonly used in the Indian traditional system of medicine

METHODS AND MATERIALS

Collection and processing of plant material

The fully grown flowers of *Butea monosperma* were collected from the local area and taken care for its freshness, healthy and free from any deformation. These flowers were dried at room temperature then blended into powder by mixture blender which then passed from the sieve to get the equal size particles. The powder should be

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aseptically kept in air tight container at the moisture free place.

Soxhlet extraction of flowers

For the extraction of flowers the selection of solvents is done with care to meet extrability and regulatory criteria. Depending upon the solubility methanol was selected for the extraction procedure.

100 gm of powder is accurately weighted and is transferred to the cup made up of 'Whatman filter paper' and placed into the extraction thimble. 500 ml of methanol was taken in round bottom flask and heated up to its boiling point, *i.e.* 65^{0}C. The methanol gets evaporated and moved in to the condenser where it was converted in to liquid trickled in to the extraction chamber containing the plant material. At the end of the extraction process, the flask containing the methanolic extract was removed and it was condensed at 50^{0}C in water bath for overnight. The weight of extract was measured and percentages of yield of the plant material were calculated. The extract was stored at 4^{0}C for further work.

Isolation of test organisms

Pure cultures of the test organisms used for antibacterial activity were isolated from the water and soil sample by using selective media. The characterization of the test organism was done by using IMVIC test. All the test organisms were cultured on nutrient agar slant. The cultures were maintained by sub-culturing periodically and preserved at 4^{0}C prior to use. (Alzoreky and Nakahara, 2003).

Screening for antibacterial activity

All the test organisms were screened for the antibacterial activity against methanolic extract of *Butea monosperma* by agar well diffusion method. With the introduction of variety of antimicrobials it becomes necessary to perform the antimicrobial susceptibility test. For this the antimicrobial agent was allowed to diffuse out into the medium and interact in a plate freshly speeded with the test organism.

Stock solution of methanolic extract was prepared to carry out the antimicrobial activities against selected cultures for the further process. For the preparation of the stock solution 1 gm of methanolic extract was accurately weighted and dissolved in 10 ml of DMSO; giving concentration of the stock solution as 100 mg/ml. this solution is then centrifuged and supernatant liquid was collected in a separate test tube, covered with paraffin wax and stored at 4^{0}C for further use.

Agar well diffusion method

The Muller-Hinton agar plates for the bacteria were prepared, 0.1 ml of fresh 18 hours old broth culture was spread on the respective media. After spreading the culture, wells of 6 mm in diameter was made at the centre of the plate by using sterile cork borer. The wells were open with the help of sterile forceps. Then 100 µl of stock solution was added by using micropipette in each well. The final concentration in the well was 10 mg/ml. The extract was allowed to diffuse; hence the prepared plates were kept in deep fridge.

After this; plates were incubated at 37^{0}C for 24 to 48 hours. The zone of inhibition was measured in millimeter and recorded. The diameter of the zone of inhibition around each well was taken as measure of antibacterial activity. Each experiments was carried out in triplicates and mean diameter of the inhibition zone was recorded.

Phytochemical screening

The methanolic extract of flowers was screened for the phytochemical content by using different chemical test for each component. It also used for the phytochemical test to detect the presence of alkaloids, tannins, saponins, flavonoids, cardiac, glycoside, anthraquinone and steroids according to standard method.

RESULTS AND DISCUSSION

Table 1 shows agar well diffusion method for demonstration of antimicrobial activity of methanolic extract of *Butea monosperma* against gram negative bacteria. The zone inhibition around the well observed for gram negative bacteria varies from 23 mm to 28 mm in diameter with highest for *Klebsiella pneumoniae* at 28.7 mm. Results presents that the bacteria are sensitive to methanolic extract of flowers. Table 2 indicates the agar well diffusion method for demonstration of antimicrobial activity of methanolic of the plant against gram positive bacteria. The zone of inhibition around the well observed for gram positive bacteria varies from 20 mm to 25 mm in diameter with highest for *Streptococcus faecalis* at 25.6 mm. Result shows that the bacteria are sensitive to methanolic extract of flowers.

Similarly, Singh and Sahu (2012) observed that methanol extracts of flowers of *Butea monosperma* were inhibitory to *Escherichia Coli* and *Pseudomonas aeruginosa* and methanol extracts was found to more inhibitory in comparison to chloroform and acetone. Lohitha (2010) observed that methanolic extract and aqueous extract of *Butea monosperma* has shown significant effect at 1000 mg/ml against *Escherichia coli* (26 mm), *Pseudomonas aerogenosa* (23 mm) and *Bacillus cereus* (25mm). Ahmad and Khan (2012) found that the MIC value of methanol extract of flowers against *Staphyloccus aureus*, *Bacillus subtilis* and *Escherichia Coli* was 2 mg/ml and for *Pseudomonas aeruginosa* was 3 mg/ml. Poornachandar and vennela (2014) shown that the chloroform extract of *Butea monosperma* recorded inhibition zone of 17 mm against cocci and 18 mm against bacilli, the aqueous extract offered inhibition zone of 20 mm against cocci and 22 mm against bacilli and the methanol extract recorded inhibition zone of 6 mm against bacilli.

Table 1. Antimicrobial activity of methanolic extract of *Butea monosperma* against gram negative bacteria (Zone of inhibition in diameter)

S. No.	Test Organism	Zone of Inhibition
1	*Salmonella typhi*	25.3 mm
2	*Pseudomonas aerogenosa*	27.6 mm
3	*Escherichia coli*	27.2 mm
4	*Shigella flexneri*	23.8 mm
5	*Vibrio cholerae*	26.4 mm
6	*Enterobacter aerogenes*	24.5 mm
7	*Klebsiella pneumoniae*	28.7 mm

Table 2. Antimicrobial activity of methanolic extract of *Butea monosperma* against gram positive bacteria (Zone of inhibition in diameter)

S. No.	Test Organism	Zone of Inhibition
1	*Bacillus subtilis*	20.8 mm
2	*Bacillus megaterium*	23.5 mm
3	*Bacillus fusiformis*	22.2 mm
4	*Streptococcus faecalis*	25.6 mm
5	*Streptococcus pyogenes*	23.4 mm
6	*Streptococcus pneumoniae*	25.3 mm
7	*Staphyloccus aureus*	24.7 mm

Table 3. Phytochemical screening of methanolic extract of *Butea monosperma* flowers

S. No.	Phytochemical constituents	Methanolic extract
1	Alkaloids	+
2	Saponins	-
3	Tannins	+
4	Steroid	+
5	Flavonoids	-
6	Anthraquinones	+
7	Glycosides	-

Rajput *et al*. (2011) found that the methanol extract of *Butea monosperma* leaves shows inhibition against *Pseudomonas aeruginosa* (14.3 mm), *Bacillus subtilis* (7.3 mm), *Staphyloccus aureus* (15.3 mm) and *Klebsiella pneumonia* (15.8 mm). Dhale *et al*. in (2010) observed that the methanol extract was found to be more effective against *Bacillus subtilis*, and *Staphylococcus aureus* (13 mm at 100 mg/ml). Gurav *et al.* in (2008) reveled that MIC value of petroleum ether extract against gram positive strains was 300 ¼g/ml and that of alcoholic extract was 200 ¼g/ml. Tambekar and Khante in (2010) reported that In vitro control capacities of the aqueous extract of *Butea monosperma* on *Escherichia coli*, *Staphyloccus aureus*, *Enterococcus spp*., *Salmonella typhi*, and *Shigella flexneri* were significant. Acharyya *et al.* in (2009) recorded the antimicrobial activities of the aqueous extract of the plant against the destructive enteric pathogen, Vibrio cholerae at the level of 4 mg/ml.

In the present investigation phytochemical screening of methanolic extract of *Butea monosperma* shows the presence of five phytochemical out of seven for which screening has performed. The methanolic extract of flowers shows the presence of Alkaloids, Tannins, Steroids and Anthraquinones. While the Saponins, Flavonoids and Glycosides were found to be absent in the methanolic extract (Table 3). In similar studies, Lohitha (2010) detected the presence of alkaloids, saponins and tannins from methanolic extract of bark of *Butea monosperma.* Rajput *et al.* (2011) reported the presence of steroids, terpenoids and glycosides from the chloroform extract of leaves of *Butea monosperma*. Sahu and Padhy (2013) found that the methanolic extact of *Butea*

monosperma leaves contains the presence of alkaloids, favonoids, sapononis and tannins.

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