ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Antimicrobial Activity and Spectral Characterization of Flower of *Butea monosperma*

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ABSTRACT

Butea monosperma is extensively used in Ayurveda, Unani and Homeopathic medicines. Different parts of the plants are useful in filariasis, night blindness, helminthiasis, diarrhea, dysentery, sore throat, snake bite, astringent, diuretic, treatment of liver disorders. The flower of *Butea monosperma* was dissolved in methanol in the ratio of 3:1 (methanol: powdered flower). The methanolic extract of flower was directly chromatographed over silica gel column eluted with solvent petroleum ether, chloroform, acetone and methanol, according to their increasing order of polarity. The extracts were characterized on the basis of various spectral technique such as IR, ^{1}H NMR, and mass spectroscopy. Antimicrobial activity of various extract of *Butea monosperma* was evaluated against some pathogenic strains. The antibacterial study performs against to bacterial species via; Escherichia coli and pseudomonas aeruginosa. The methanol extracts of flower of *Butea monosperma* exhibited varying level of antibacterial activity, with minimum inhibitory concentration (MIC) of 2 mg/ml against both bacteria. The methanol extract was found to be more active than the other extract against both the bacteria. The antifungal activity of these extracts was also performed against to alternaria brassica fungal strain. The methanol and acetone extracts showed moderated as well as significant activity against the fungal strains.

Keywords: Flower, Antimicrobial, Spectral characterization, In-vitro, IR

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ISSN: 0975-8585

INTRODUCTION

Herbs and minerals used in Ayurveda were later described 700 medicinal plants, 64 preparation form mineral sources and 57 preparations based on animal sources [1]. The use of herbs to treat disease is almost universal among non – industrialized societies. A survey of herbalists in the UK found that many of the herbs recommended by them were used traditionally but had not been evaluated in clinical trials [2]. Active compounds currently isolated from the higher plants and widely used in modern medicine today, 80 percent show a positive correlation between their modern therapeutic use and traditional use of the plants from which they are derived [3]. The phytochemical interaction and trace components may alter the drug response in ways that cannot currently be replicated with a combination of a few putative active ingredients [4]. Pharmaceutical researchers recognize the concept of drug synergism but note that clinical trials may be used to investigate the efficiency of a particular herbal preparation, provided the formulation of that herb is consistent [5]. Many herbs have shown positive results in-vitro, animal model or small scale clinical tests but many studies on herbal treatments have also founds negative results [6]. All the natural plants contain chemical constituents of terpenes, flavonoids, alkaloids, steroids and glycosides etc.

Butea monosperma is also extensively used in Ayurveda, Unani and Homeopathic medicine and has become a cynosure of modern medicine. The plant is regularly used by the rural and tribal people in curing various disorders [7]. Butea monosperma is commonly known as flame of forest belongs to the family of fabaceae [8]. The plant *Butea monosperma* is known to possess numerous medicinal properties and almost all parts of the plants have been used since decades in medicines and for other purposes [9]. The economic uses such as leaves are used for making platters, cubs, bowls and beedi wrappers. Roots are useful in filariasis, night blindness, helminthiasis, piles, ulcer and tumors [10]. Tiwari et al [11] has investigated the hepatoprotective activity of the stem bark extract of *Butea monosperma* against carbontetrachloride induced liver damage. The stem bark of *Butea monosperma* is also useful in antitumor, antiulcer, antifungal, antidiarrhoeal activities and indigenous medicine for the treatment of dyspepsia, sore throat and snake bite [12]. Leaf of *Butea monosperma* (Lam.) Taub (palas) belonging to the family of leguminoceae is appetizer, very astringent, carminative, anthelminitic, aphrodisiac, tonic, lessen inflammation and lumbago, cures boils and piles. The fruit and seeds of this plant are bitter and oily, anthelmintic, useful in piles, eye diseases and inflammation [13]. The study of antihyperglycemic and antioxidant potential of hydroethanolic extract of *Butea monosperma* seeds and its active constituents have been investigated by Sharma and Garg [14]. The anticancer activity of ethanolic extract of leaves of *Butea monosperma* has recently reported [15]. Flowers are useful in diarrhea, astringent, diuretic, depurative and tonic [16].

In the above point of view we have isolated various extracts of flower of *Butea monosperma* such as chloroform extract (C1), acetone extract (C2) and methanol extract(C3) by chromatographic techniques and the extracts were characterized by IR, ^{1}H NMR and mass spectroscopy. The microbial activity of these extracts has been studied against bacteria and fungal strain.

ISSN: 0975-8585

MATERIALS AND METHOD

Plant Materials

The flowers of *Butea monosperma* were collected from local market, Kanpur, INDIA. The flower was identified by Prof. Kausal Kumar, CSA University, Kanpur. Flower of the plant were cleaned with distilled water, dried and crushed in mixer grinder and the grinding was performed in a hygienic condition.

Extract Preparation

The powder material of flower was dissolved in methanol in the bucket. The ratio of the methanol and powdered flower were 3:1, respectively. It was stirred about one hour to make sure that the compound gets dissolved in solvent and it was left for 24 hours. The extract was taken out and filtered using sterile filter paper and concentrated under reduced pressure for the crude products. A gummy solid was directly chromatographed over silica gel column and eluted with solvent petroleum ether, chloroform, acetone and methanol in their increasing order of polarity.

Analysis and Measurements

(a) Spectral Analysis

The extracts were characterized by the various spectral techniques such as IR, ^{1}H NMR and mass spectrophotometer. The infra red spectra were recorded on FT-IR spectrophotometer, brucker (vertex 70) using KBr pallets in a range of 4000-400cm^{-1}. Zeol 400 MHz spectrophotometer was used for recording the ^{1}H NMR spectra using $CDCl_3$ as solvent and TMS as internal standard. Mass spectra were recorded on a Jeol SX 102/Da-600 mass spectrophotometer.

(b) Test Organisms

The test microorganism used for the antibacterial activity was performed on broth and nutrient agar media which contains 0.5g peptone, 0.5g NaCl, 0.3g beef powder extract and 1g peptone, 1g NaCl, 0.6g beef powder extract, 4.0g agar, respectively. Bacteria were cultured over night at 28^{0}C for 24 hour in Muller Hington broth inoculums. Sterile Petri disc with a diameter of 7mm plats were prepared by pipetting 100µL volume of stock solution of extract (2mg/ml) on to sterile blank plates. The plates were air dried and stocked solution at 4^{0}C, used within two days, a plate containing solvent extract was applied to incubated plates by using flamed forcipes. Antibacterial activity of extracts of flower of *Butea monosperma* were evaluated by plate method using 100µL of suspension containing 10^{8} CFU/ml of bacteria spread on Mullar Hington Agar medium. The extracts were dissolved in CH_3OH at a concentration of 50mg/ml. The disc impregnated with 100µL of extracts placed on seeded agar and the disc plates were incubated at 28^{0}C for 24 hours depending on the diameter of zone inhibition

ISSN: 0975-8585

formed around the plates. The fungi were isolated from the infected part of their respective hosts via Alternaria brassica, culture from laboratory strain. Fungi were cultured on potato dextrose agar (PDA) medium at 25 ± 2^0C, the culture were purified by single spore germination on PDA slant with composition of 250g peeled potato, 20g dextrose, 20g agar and 100 ml distilled water, slants and stored at 4^0C for further use. The purified compounds in respective solvent were tested for their antifungal activity by silica gel TLC method. Different concentration of compounds were prepared .20µL of each concentration was spotted on TLC plates, dried at room temperature and overspread with spore suspension (1 x 10^7 spore/ml) of test fungi in Czapak –Dox medium. The plates were incubated in humid chamber at 25 ± 2^0C for 3 days until the growth of the fungus become visible. A control plates spotted with the corresponding organic solvent was run in parallel. The minimum inhibitory concentration (MIC) was defined as the minimum concentration at which on fungal growth was observed that is showing a clear zone of inhibition.

RESULTS AND DISCUSSION

IR Spectra

The IR spectrum (Fig.1) of extract C_1 exhibited a band at 3445cm^{-1} which clearly verified the presence the hydroxyl group. Two strong peaks at 2962cm^{-1} and 2924cm^{-1} indicated the presence of ν(CH_2) group. The appearance of absorption of frequency at 1737cm^{-1} showed the presence of carbonyl group. Further a peak at 1609cm^{-1} was clear evidence of the presence of υ(C=C) group. The IR spectrum (Fig.1) of extract C_2 showed a band at 3391cm^{-1} indicated the presence of hydroxyl group. The peak at 2925cm^{-1} indicated the presence of ν(CH_2) group. The appearance of absorption of frequencies at 1609cm^{-1} showed the presence of carbonyl group. The υ(C=C) was confirmed by the presence of peak at 1514cm^{-1}. In the IR spectrum (Fig.1) of C_3 extract, the band at 3384cm^{-1} indicated the presence of ν (OH) group. The appearance of absorption of frequency at 2922cm^{-1} showed the presence of ν(CH_2) group. The carbonyl group was confirmed by the presence of peaks at 1609cm^{-1} . The appearance of peak at 1514cm^{-1} showed the presence of υ(C=C) group in C_3 extract. The IR spectral data of extracts are shown in Table 1.

Table 1 Infrared spectral data of the extracts (cm^{-1})

Extracts	υ(OH)	υ(C-H)	υ(C=O)	υ(C=C)
C_1	3445	2962	1737	1609
C_2	3391	2925	1609	1514
C_3	3384	2922	1609	1514

NMR Spectra

^{1}H NMR spectra (Fig.2) of the extract C1 showed a singlet peak at 8.11ppm indicates the hydroxyl proton attach with benzene ring. The ring proton was confirmed by the appearance of the peaks at 6.68ppm to 7.53ppm. The appearance of peaks at 4.45ppm indicated the presence of olefinic group. The C2 extract (Fig.2) exhibited low field signal for the hydroxyl proton at

ISSN: 0975-8585

7.63ppm. Signal for the ring proton of C2 extract was observed between 6.66ppm and 7.53ppm. In the region of 5.32ppm were assigned to chemical shifts for proton of the olefinic group. In ^{1}H NMR spectrum (Fig.2) of the extract C3, the presence of the aldehydic proton was characterized as a singlet at 9.1ppm. The peak at 8.79ppm was assigned for the hydroxyl group and the peak due to benzene ring protons was obtained in the form of multiplet at 7.54ppm, whereas peak at 5.49ppm was attributed for the olefinic group. The ^{1}H NMR spectral data of extracts are shown in Table 2.

Table 2 ^{1}H NMR data of extracts δ (ppm)

Extracts	^{1}H NMR spectrum, δ(ppm)
C_1	8.11(1H,s,-OH), 7.53(1H,d,Protons of benzene ring), 6.68(1H,d, Protons of benzene ring), 4.45(1H,d,C=C).
C_2	7.63(1H,d,-OH), 6.66-7.53(1H,d,Protons of benzene ring), 5.32(1H,d, C=C).
C_3	9.1(1H,s,-CHO), 8.79(1H,d, -OH), 7.54(2H,m,protons of benzene ring), 5.49(1H,d,C=C).

Mass Spectra

The mass spectrum of extract C1 showed the highest molecular ion peak at m/z value 272 which corresponds to the molecular weight of the extract C1. The m/z value 272 also corresponds to extract C2, which was exactly match to the extract C1. The structures of extract C1 and extract C2 were confirmed by the same molecular weight. The mass spectrum of extract C3 showed the m/z value 585 due to attachment of two additional glucose moieties with both side of benzene rings.

Biological Studies

Table 3 Antibacterial activity of extracts

Bacterial Species	Chloroform extract	Acetone extract	Methanol extract
Escherichia Coli	+	+	+
Pseudomonas Aeruginosa	-	-	+

Table 4 Antifungal activity of extracts

Fungal Species	Chloroform extract	Acetone extract	Methanol extract
Alternaria Brassicea	-	+	+

The sensitivity of bacterial strains to various extracts (Table 3) in the chloroform, acetone and methanol were inhibitory to the test organisms Escherichia coli and Pseudomonas aeruginosa. Methanol extracts of flower of *Butea monosperma* were observed inhibitory to Escherichia Coli and Pseudomonas aeruginosa and methanol extracts was found to more inhibitory in comparison to chloroform and acetone against Escherichia Coli. No inhibitory activity was observed in the chloroform and acetone extracts against Pseudomonas aeruginosa. The antifungal activity of the different extracts (Table 4) was examined against fungal strain Alternaria brassicea organism by measuring zone of inhibition. The acetone and methanol

ISSN: 0975-8585

extracts showed sensitivity at minimum concentration while the chloroform extract showed no sensitivity against Alternaria brassica strain.

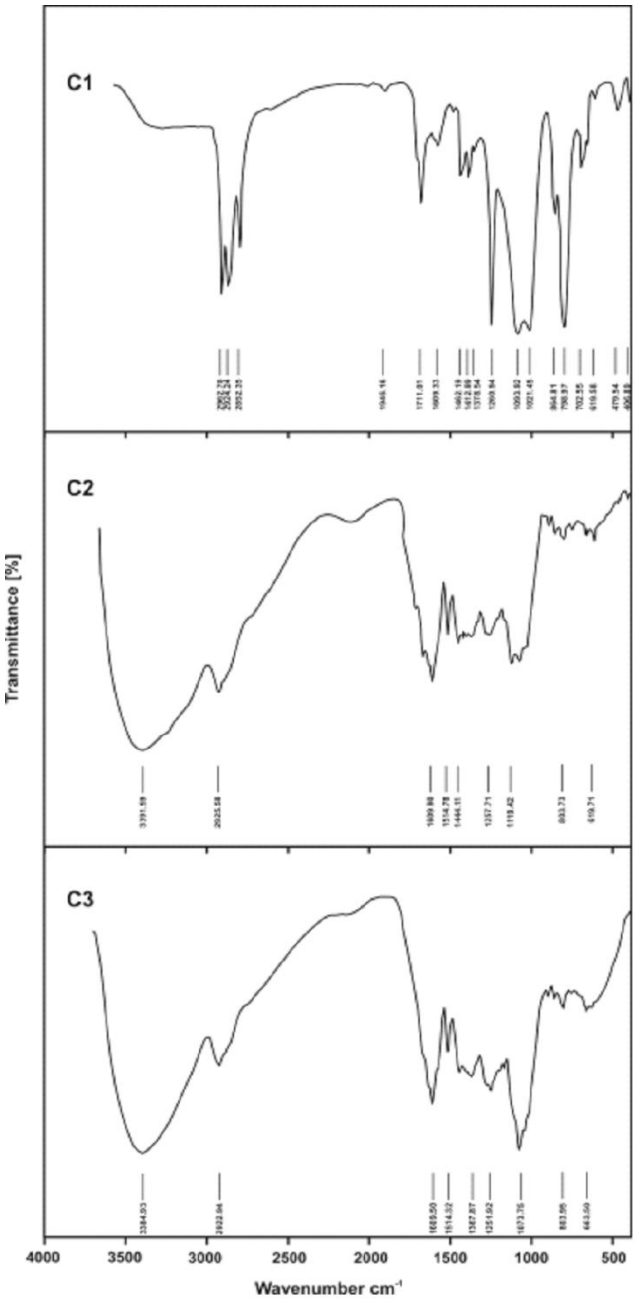


Fig. 1. IR Spectra of extracts C1, C2 and C3

ISSN: 0975-8585

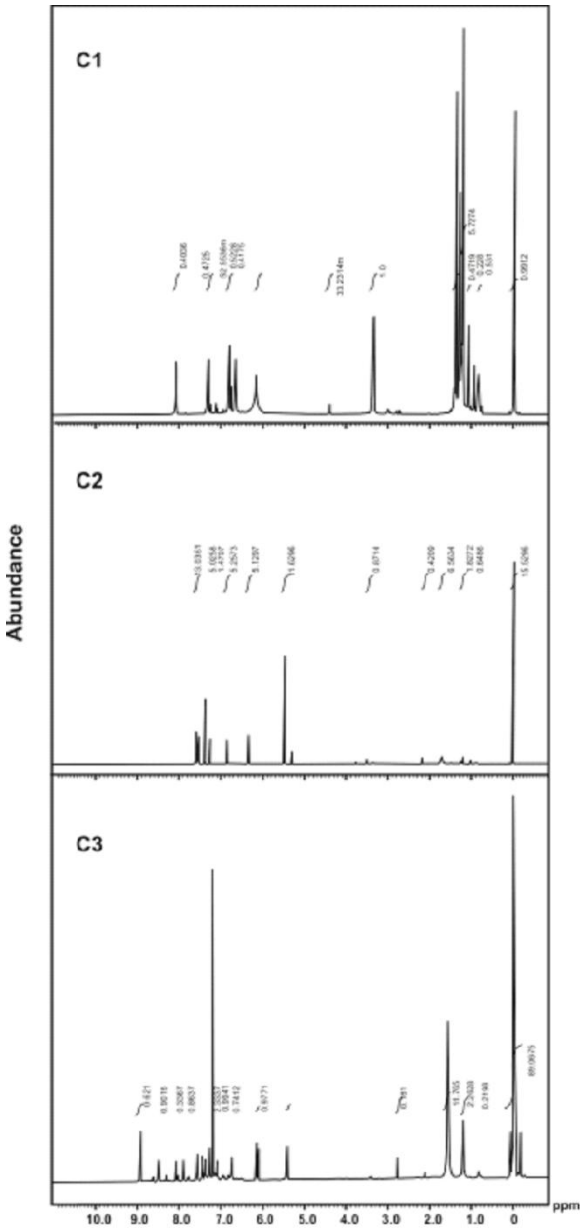
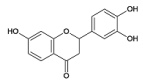
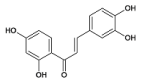


Fig. 2. 1H NMR Spectra of extracts C1, C2 and C3

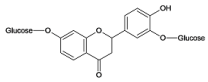
ISSN: 0975-8585



C1



C2



C3

Fig 3 Proposed Structure of Extracts

CONCLUSION

The functional groups detected in the isolated extracts C1, C2 and C3 were ν(OH) ν(CH_2), and ν(CO). Different protons of glucose, benzene ring, olefinic group and methylene ring chain were observed form the ^{1}H NMR study to support the structures (Fig.3). The molecular weights of the extracts were calculated from the mass spectra to correlate the structures which were exactly match with the molecular weight of proposed structure. The chemical constituents like saponins, alkaloids and carbohydrates are responsible to antimicrobial activity of the crude drug. The presence of these bioactive components in the crude drugs has been linked to their activities against diseases causing microorganism and also offering the plants themselves protection against infection by pathogenic microorganism. The extraction of biological active compounds from the plant materials depends of the type of solvent used in the extraction procedure. Most of the antimicrobial active compounds that have been identified were soluble in polar solvents. The methanol extracts of *Butea monosperma* flower was chromatographed over silica gel column eluting with different organic solvents with their increasing order of polarity to separate the components in each solvent for their antimicrobial activity. The sensitivity of bacterial strain to various extracts revealed that the methanol extracts was found to be more active than the other extracts against both the bacteria. The methanol and acetone extracts showed moderated as well as significant activity against the fungal strains.

ACKNOWLEDGEMENTS

Authors are thankful to the Director, University Institute of Engineering & Technology, C.S.J.M. University, Kanpur for providing laboratory facilities.

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