



Phytochemical Activities of *Artemisia absinthium* and *Butea monosperma* Plant Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. Author FW designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SY and QA managed the analyses of the study. Author AM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

With the developing time, there is a tremendous need to deliver highly potential antimicrobial drugs and treatment from different herbaceous plants because plants are now days commonly used to provide resistance against disease-causing pathogens. This study has been done by using agar well diffusion method. The bacterial along with fungal strains were collected and were cultured on agar plates. Afterwards, these plates were put in incubate for 24 hours at 37⁰C temperature. Within this duration, the zones were developing all around the plates, wherever plant extracts were poured. The activities of microorganisms were measured by using inhibition diameter zones. The inhibition zones were shown clearly on petri plates. Antimicrobial activities of plant extracts of *Artemisia absinthium* and *Butea monosperma* tested against bacterial and fungal strains viz. *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli* as well as fungal strains were also used viz. *Aspergillus niger* and *Fusarium oxysporum*.

Keywords: *Artemisia absinthium*; *Butea monosperma*; phytochemicals; antibacterial; antifungal.

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1. INTRODUCTION

The development of drug resistance in human pathogens in opposition to normally used antibiotics has necessitated a search for new antimicrobial substances from other resources which include flora. Plants used for classic medicinal drug contain a huge variety of materials that can be used to treat continual as properly as infectious sicknesses. The substances that may both inhibit the boom of micro-organisms or kill them are considered as an important agent for growing new pills for medication of diverse infectious diseases. The use of medicinal plant life as traditional medicines is widely known in rural areas of many developing international locations [1-3]. Recently the inhibitory consequences of numerous agricultural plant extracts on the growth of many bacteria in subculture were studied in our laboratory [4,5]. *Artemissia absinthium* (compositae/ Asteraceae), called "Wormwood," whole plant and leaves are broadly used. It is an aromatic, diuretic, sour herb that has anti-inflammatory outcomes, and acts as a tonic for the liver, digestive machine, and nerves. It stimulates the uterus and expels intestinal worms. It is used internally for digestion, bad appetite, gall bladder complaints, and roundworms, also externally for bruises and bites. It is taken in small doses for short-term remedy; it isn't always given to children or pregnant women [1,6,7]. Formerly in European countries, an alcoholic liqueur known as "Absinthe" turned in to crafted from this plant, however nowadays due to the fact of the continual intoxication and apprehensive disorder, this drink id forbidden [8-10].

Artemissia absinthium is essentially grown in Europe, Asia and Turkey too. It provides a green-blue essential oil. The principal additives of this vital oil are as follows: a-fenchene, b-myrcene, endo-bornyl acetate, and b-pinene [11]. Many *Artemissia* species have function fragrance or flavor, based totally on monoterpenes and sesquiterpenes that in lot of instances are the reasons for their application in people medication [8]. In traditional medicine, there are many natural crude tablets which have the ability to deal with many diseases and disorders considered one of them is *Butea monosperma* popularly called "dhak" or "palas". They are considered one of the most important households of flowering flora, with 630 genera and 18,000 species. This is medium sized tree that is broadly dispensed all through India,

Burma and Ceylon extending in the North West Himalayas [12].

It is one of the most stunning tree has been placed to a few beneficial cause. *Butea monosperma* is extensively utilized in Unani and Homeopathic medicinal drug and has emerged as a cynosure of cutting-edge medicine. The flora of this genus is widely recognized for its coloring matters. Commonly *Butea monosperma* is used as tonic, astringent and diuretics. Roots are useful in night time blindness, piles, ulcer and tumours. Flowers are useful in diarrhea, astringent, diuretic [13]. The stem bark is an indigenous remedy for the treatment of dyspepsia, diarrhea, dysentery, ulcer, sore throat and snake chunk. Besides medicinal makes use of it is also having financial use including leaves is used for making platters, cups, bowls and beedi wrappers. Bark fibres are used for making cordage. It is reasonable price board wooden. Wood pulp is suitable for newsprint production. *Butea* is also a host to the *Kerria lacca* also know as Lac insect, which produces herbal lacquer [14]. The present study was conducted to evaluate the *Artemissia absinthium* and *Butea monosperma* extracts for antioxidants, antibacterial and antifungal activities.

2. MATERIALS AND METHODS

This experiment was done at the Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan.

2.1 Specimen Collection

The samples of plants including *Artemissia absinthium* and *Butea monosperma* was collected from the market. The samples were in their original form. They were crushed fine powder by using mortar and pestle.

2.2 Plant Extraction

A total of 6 extracts were prepared from both the plants. For preparing the extract three different solvents were used in it viz. acetone, n-hexane and aqueous or distilled water. The crushed sample was measured on digital balance. The 20 grams of each sample was taken and soaked in 100 ml of each solvent in 250 ml of conical flask. After adding the sample and solvents in flask, the flasks were continuously stirred one by one so that both objects simultaneously mixed together. Afterwards flasks were tightly covered with aluminium foil and the flasks were left for 48

hours with continuously shaking after regular intervals. After 2-3 hours the supernatant from the flasks were removed and remaining extract was filtered through filter paper.

2.3 Rotary Evaporator

The rotary evaporator is widely used to separate both the solute and solvents from each other. We passed the sample from rotary evaporator one after another. For the samples of acetone and n-hexane we put temperature of about 60°C and for samples of distilled water we put temperature at 100°C as it is boiling point of water. The resulted sample is stored in eppendorf tubes and stored it in refrigerator.

2.4 Preparation of Media

The media was prepared by using 5.7 g Agar and 7.6 grams of Nutrient broth in flasks. The flasks were continuously stirred to remove any lumps; following it flasks were heated on a Bunsen burner till bubbles were appeared. After cooling it, the mouths of flasks were covered with aluminium foil and autoclaved prepared media. After sterilization of media, it was poured in to sterilized Petri plates [15].

2.5 Agar Well Diffusion Methods

The strains (*Pseudomonas*, *E. coli* and *Bacillus*) and for fungal (*Fusarium* and *Aspergillus*) had then streaked on Petri dishes in which nutrient media was already added. For streaking we used loop and Bunsen burner for removal of any kind of contamination. By using loop, each strain was carefully streaked on prepared media plate so that we get appropriate inhibition zones. The wells were made in plates and plant extracts were also introduced inside these wells with the help of micropipettes. The plates were tightly wrapped with the use of parafilm and left in incubator for 24 hours at 37°C temperature. Antimicrobial activities of both bacteria and fungus were calculated through the diameter of inhibition zones is measured in millimeters produced against pathogens. The same experiment has been repeated 2 times [16,17].

2.6 Preparation of 1M DPPH Solution

For preparing 1M of DPPH solution, 4mg of DPPH has been taken along with 100 ml of methanol has included in jar. Flask was shaken

after equal intervals so they can mix well. After stirring flasks mouth were covered with parafilm and put in cool conditions. Following it samples were put in water bath for 2.5 hours [18].

2.7 Centrifugation

Afterwards samples were removed from water bath and again stirred for mixing the solvent completely. Then the samples were put in centrifuge tubes for 15 minutes at 6000-8000 rpm. Centrifugation is a way of keeping apart molecules having one of kind densities by using spinning them in solution around an axis (in a centrifuge rotor) at high velocity. After that specific time tubes were removed from centrifuge and the upper solution known as supernatant was removed with the help of filter paper.

Some series of solution has been prepared by utilizing extracted solution and methanol of 1ml, 2 ml, 3 ml, 4 ml and 5 ml. 1 ml of each sample has been taken and 3 ml of DPPH solution is included in each sample. After that every solution has placed away with 99% methanol up to 10ml. Then the prepared sample was kept a side for about 30 minutes. After that, the sample is finally passed through spectrophotometer assay.

2.8 Statistical Analysis

The numerical data was recorded for inhibition zones and analyzed for inhibition percentage.

3. RESULTS

3.1 Antibacterial Activity Test Results

Antibacterial activity of acetone and n-hexane from the samples of herbaceous plants *Artemisia absinthium* and *Butea monosperma* were tested against bacterial strains of *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis*.

The results obtained from this study are mentioned below.

3.2 Anti fungal Activity Test Results

Antifungal activity of herbaceous plants viz. *Artemisia absinthium* and *Butea monosperma* were tested against fungal strains of *Aspergillus niger* and *Fusarium oxysporum*. The results obtained from this study is mentioned below.

Table 1. Antibacterial activity of zone of inhibition (mm) produced by acetone and n-hexane extracts of *Artemisia absinthium*

Bacterial strains	Dose 2.5 µl	Dose 5 µl	Control Conditions
<i>Pseudomonas Aeruginosa</i>	5	8	0
<i>Escherichia coli</i>	3	6	0
<i>Bacillus subtilis</i>	8	10	0

Table 2. Antibacterial activity zone of inhibition (mm) produced by acetone and n-hexane extract of *Butea monosperma* against various bacterial species

Bacterial strains	Dose 2.5 µl	Dose 5µl	Control condition
<i>Pseudomonas aeruginosa</i>	9	3	0
<i>Bacillus subtilis</i>	12	8	0
<i>Escherichia coli</i>	4	7	0

Table 3. Antifungal activity zone of inhibition (mm) produced by acetone and n-hexane extracts of *Artemisia absinthium* against various fungal strains

Fungal strains	Dose 2.5 µl	Dose 5µl	Control Conditions
<i>Aspergillus niger</i>	5	8.9	0
<i>Fusarium oxysporum</i>	4.2	5.3	0

Table 4. Antifungal activity zone of inhibition (mm) produced by acetone and n-hexane extracts of *Butea monosperma* against various fungal species

Fungal strains	Dose 2.5 µl	Dose 5µl	Control Conditions
<i>Aspergillus niger</i>	6.6	7.5	0
<i>Fusarium oxysporum</i>	3	4.3	0

Table 5. Antioxidant activity produced by methanol extracts of *Artemisia absinthium*

Obtained extracted Solution (ml)	Concentration (mg/ml)	Absorbance (A)	Inhibition %
Control	0	0.105	
1	3	0.260	6.3
2	6	0.245	03
3	12	0.215	17.57
4	15	0.203	20.99
5	18	0.195	28.88

Table 6. Antioxidant activity produced by methanol extracts of *Butea monosperma*

Obtained extracted Solution (ml)	Concentration (mg/ml)	Absorbance (A)	Inhibition %
Control	0	0.300	
1	2	0.285	5.76
2	4	0.270	9.95
3	6	0.250	16.65
4	12	0.215	37.53
5	18	0.203	32.52

3.3 Antioxidant Activity Test Results

Antioxidant activity of *Artemisia absinthium* and *Butea monosperma* was tested by using DPPH method. The results obtained are given below.

4. DISCUSSION

Acetonem n-hexane and aqueous or water extracts of different plants viz. *Artemisia absinthium* and *Butea monosperma* have shown

tremendous activity against some pathogenic diseases. Whereas, aqueous or water extracts show no activity against these plants. As the different studies affirmed that *Artemisia absinthium* are the viable inhibitors of bacterial development. The extracts of plants proved the different degrees of activity against pathogenic organisms. The acetone grew to become out to be more outstanding in opposition to all examined lines of microscopic organisms whilst contracted with n-hexane extracts. This can be due to limitations of acetone to extracts an extensively higher as compared with n-hexane which could probably have extracted much less portions of the components. The extracts confirm the higher activities towards *Bacillus subtilis* and *Pseudomonas aeruginosa* contracted with the strains of *Escherichia coli*. As an *Artemisia absinthium* incorporates tannin, flavonoids, polyphenolic mixes glycosides and herbal acids. The antimicrobial activity of plants is most possibly as a consequence of polyphenolic mixes. It has been determined that the polyphenolic mixes are answerable for the antibacterial activity of the plant [19,20].

The aqueous, acetone and n-hexane extracts of *Butea monosperma* has established great measured activity. In comparison of *Pseudomonas aeruginosa* and *Escherichia coli* (15mm, 8mm) and *Bacillus subtilis* (6mm) it might be finished up from the influences that *Butea monosperma* plant extracts have antimicrobial activity in comparing to diverse utilized living beings. A part of the extracts (acetone, n-hexane) were proving to extra effective than conventional antimicrobial to battle the pathogenic microorganisms studied [21-23]. This likely approach the compound chargeable for antimicrobial activity is available in each extract at one of type concentration. The extracts had been observed to be compelling against *Escherichia coli* whole evaluate with *Bacillus subtilis*. The phyto-constituents within the extracts may be responsible for the antimicrobial activities [24]. The in-vitro examine of *Butea monosperma* on various organisms may additionally help with finding better processes of anti infection substances that may work precise operators for sickness. The technique has spread the chance of making use of this plant development for human utilization for extra pre determination use [25-29]. An antioxidant is mainly crucial substances that have the potential to tie down the human body from harm due to unfastened extremist expanded oxidative stress. The results obtained from DPPH are in

concurrence with the absorbance material determined for pattern. DPPH is a strong free method that have generally been applied to check the antioxidant potential of domestic gown extracts. The antioxidant belongings of the function are assessed with the aid of approach of estimating the absorbance of materials. The absorbance and cell activity are inversely proportional to each other [30-33]. Methanolic extracts of natural plants have various concentrations from 0 to 2µg/ml. they have been verified huge absorbance of DPPH scavenging. i.e., from 0 to 0.300 and inhibition percentage commenced from 5.6 to 32.54 respectively. At a similar grouping of samples of *Artemisia absthinum* vivek et al studied 59.80% inhibition zones.

5. CONCLUSION

Organic solvent extracts of *Artemisia absinthium* and *Butea monosperma* had been powerful towards bacterial and fungal species such as *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, *Aspergillus niger* and *Fusarium oxysporum* isolates. Acetone and n-hexane were the most effective solvents for this manner. On contrasting the consequences of antibiotics, it is under observation that huge majority of tests have been profoundly impervious to almost all antibiotics. The plant phyto-chemicals hindered bacterial activities more successfully than the commercially available antibiotics. Extraction of herbaceous plants using various chemicals can be the perfect reaction to the growing problems of microbial protection from manufacturing antimicrobial. It is very well can be trusted that studies like it can enhance the utilization of compounds that could be used as medications of natural beginning.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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