Abstract
The present research work was focused on the antibacterial activity of seed extracts of Sesamum indicum. The aqueous and ethanolic extracts of seeds prepared from various extraction procedures were evaluated against a Gram positive bacteria Bacillus subtilis and a Gram negative bacterial strain, Escherichia coli by agar well diffusion method. Preliminary phytochemical screenings of the extracts were also done. Various concentrations (100 µg/ml, 200 µg/ml and 300 µg/ml) of the extracts were incorporated into the agar wells. A single dose of standard tetracycline (300 µg/ml) was also placed in the well. Both the extracts showed concentration dependant activity against the microbes. The results were analyzed based upon the readings of diameter of zones of inhibition. Both the seed extracts showed better inhibitory activity against gram negative bacteria. The test substances were compared with that of the standard drug tetracycline. The ethanolic extract showed significant activity against the test organisms than that of the aqueous extract. It was concluded that the seed extracts of Sesamum indicum showed better anti bacterial activity and further studies were suggested to isolate the active principles that were responsible for the activity.

Key words
Antibacterial, Sesamum indicum, Zone of inhibition, Bacillus subtilis, Escherichia coli, Tetracycline

Introduction
Antibacterials are the agents which are used for the treatment of infections caused by various bacterial species. The frequency of life threatening infections caused by the pathogenic microbes has increased all over the world (1). The increasing prevalence of multi-resistant strains of bacteria and the strains with reduced susceptibility to bacteria evoked the search for new infection fighting strategies (2). Plants are the sources of many potent and powerful drugs (3). Many studies were done worldwide to investigate the antibacterial property of plants as they are the cheapest and safer alternatives (4, 5, 6). Considering such potentiality of plants, a systematic investigation was undertaken to screen the locally available plant Sesamum indicum for the antibacterial activity.

Sesamum indicum, belonging to the family pedaliaceae, is an annual branching plant with 4 – 5 feet height. The shape of the leaves varies. The flowers were reddish white, single and were located in the axils of the leaves. The fruit is an oblong capsule with small oval blackish seeds. Sesame oil is used in the preparation of iodinol and brominol which were employed for the external, internal and subcutaneous use. It is also used in making some liniments, plasters and ointments. They are used well in the treatment of different cases like infantile diarrhoea, dysentery, helminth and microbial infections. The oil is said to be a laxative and is also used to promote menstruation (7). Hence the present...
study was undertaken specifically to investigate the role of *Sesamum indicum* seed extracts as antibacterials.

Materials and Methods

Plant material

*Collection and authentication of plant materials:* The seeds of *Sesamum indicum* belonging to the family Pedaliaceae were collected in the month of August, 2013 from the local areas of Guntur district, Andhra Pradesh, India. The plant material was identified and authenticated by Dr. Sreenivasa prasanna, M. Pharm., PhD, Department of Pharmacognosy, M. L. College of Pharmacy, Singarayakonda, Andhra Pradesh, India and a voucher specimen (MPESFPS – 02/13) has been deposited.

*Processing of sample:* The seeds of *Sesamum indicum* were collected, dried at ambient conditions and pulverized into fine powder which is used for the extraction process. Here maceration and soxhletation processes were employed for the extraction of seeds.

Preparation of extracts

*Preparation of aqueous extract of Sesamum indicum seeds:* The powdered dried seeds were subjected to extraction process by maceration with distilled water at room temperature for 48 hours with occasional stirring. The extract was filtered after 48 hours, then concentrated to dryness at room temperature and preserved.

*Preparation of ethanolic extract of Sesamum indicum seeds:* The powdered dried seeds were loaded into the soxhlet extractor and subjected to extraction with ethanol. After extraction, the solvent was distilled off, concentrated to dryness at room temperature and preserved.

Phytochemical analysis: The aqueous and ethanolic extracts of *Sesamum indicum* seeds were subjected to preliminary phytochemical screening by using various phytochemical tests for qualitative analysis of presence of various constituents like carbohydrates, fixed oils, glycosides, alkaloids, flavonoids, tannins, polyphenols, steroids and saponins (8, 9). The various tests include

**Test for Carbohydrates**

*a. Molisch’s test:* Aqueous or alcoholic solution of the extract was added with 10% aqueous solution of alpha napthol and shaken. Concentrated sulphuric acid was added along the sides of the tube. Violet ring at the junction of two liquids shows the presence of carbohydrates.

*b. Benedict’s test:* 5 ml of benedict’s reagent was added to 3 ml of test solution, boiled for 2 minutes and allowed to cool. Greenish yellow or red precipitate shows presence of reducing sugars.

*c. Barfoed’s test:* 2 ml of test solution was added to 2 ml of barfoed’s reagent and boiled. Brick red precipitate shows the presence of monosaccharides.

**Test for fixed oils:** The presence of fixed oils was determined by placing 5 ml of test solution in an ordinary paper. Observation of a translucent spot indicates the presence of fixed oils.

**Test for Glycosides:** A pinch of the extract was treated with glacial acetic acid and few drops of ferric chloride solution, followed by the addition of concentrated sulphuric acid. Formation of a red ring at the junction of two liquids indicates the presence of glycosides.

**Test for alkaloids:** To the extracts, dilute hydrochloric acid was added and filtered. The filtrate was treated with the extracts.

*a. Mayer’s test:* When the filtrate was treated with mayer’s reagent, potassium mercuric iodine solution, appearance of cream coloured precipitate indicated the presence of alkaloids.

*b. Hager’s test:* The filtrate when treated with hager’s reagent, picric acid, appearance of yellow coloured precipitate indicated the presence of alkaloids.

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Test for flavonoids

a. Zinc hydrochloride test: To the extract, a mixture of zinc dust and concentrated hydrochloric acid was added. Appearance of red colour after few minutes indicates the presence of flavonoids.

b. Shinoda’s test: The extract was dissolved in alcohol and to that a few magnesium turnings were added followed by concentrated hydrochloric acid drop wise and heated. Appearance of magenta colour shows the presence of flavonoids.

Test for tannins and polyphenolic compounds

a. Ferric chloride test: 5% W/V solution of ferric chloride in 90% alcohol was mixed up with the test solution. Appearance of violet colour indicates the presence of phenols.

b. Lead acetate test: 10% lead acetate solution was mixed up with the test solution. Appearance of white precipitate indicates the presence of tannins.

Test for steroids

a. Liebermann – Burchard’s test: The extract was treated with 3 ml of acetic anhydride, few drops of glacial acetic acid followed by a drop of concentrated sulphuric acid. Appearance of bluish green colour indicates the presence of steroids.

b. Salkowski’s test: The extract was treated with 3 ml of acetic anhydride, few drops of concentrated sulphuric acid. Appearance of yellow colour indicates the presence of steroids.

Test for saponins

Foam test: 1 ml of extract was diluted to 20 ml with distilled water and shaken well in a test tube. The formation of foam in the upper part of the test tube indicates the presence of saponins.

Test microorganisms

The bacterial strains which were used in the present study are Gram-positive, namely, *Bacillus subtilis* and a Gram-negative, namely, *Escherichia coli*. The strains were obtained from the Department of Biotechnology, Bapatla College of pharmacy, Bapatla, Andhrapradesh, India.

Screening of Antibacterial activity: The agar well diffusion method was used to determine the inhibitory effects of the *Sesamum indicum* seed extracts against the microbes (10). The bacterial isolates obtained were first grown in a nutrient broth for 18 hrs at 37°C. Simultaneously, nutrient agar medium was prepared and sterilized. Then it was poured into sterile petri dishes while hot and then allowed to set. After ensuring that the medium was solidified, 0.2 ml of broth culture of the bacteria was aseptically inoculated on this plate using a sterile cotton swab and allowed to dry (11). A uniform growth was ensured for accurate results. Wells of 6 mm size was made in the agar plates with the help of the sterile cork borer and these wells were loaded with different concentrations (100 µg/ml, 200 µg/ml, 300 µg/ml) of the aqueous and ethanolic extracts of *Sesamum indicum* seeds. A standard (tetracycline) solution which acts as a positive control was also placed (300 µg/ml) in an extra well in each of the dishes. Methanol was used for the dilution of the extracts as well as the standard drug. Then these plates were incubated at 38°C for 24 to 48 h. After the incubation time, the zones of inhibition were measured in millimeter diameter using a meter rule (12).

Minimum Inhibitory Concentration (MIC): The lowest concentration of the plant extract required for inhibiting the growth of the bacterial strain was considered as the minimum inhibitory concentration (MIC). The minimum inhibitory concentration for both the gram-positive and gram-negative organisms was found by agar streak dilution method (13). Stock solutions of aqueous and ethanolic extracts of *Sesamum indicum* were mixed with the known quantity of molten sterile agar media aseptically. About 20 ml of the media containing the extract was poured into each sterile petri dish and allowed to solidify. Microorganisms were then streaked one by one on the agar plate aseptically. After streaking, all
the plates were incubated at 37 ± 1°C for 24 hours and the plates were observed for the growth of the microorganism. The lowest concentration of the plant extract at which the MIC was observed and noted.

Results and Discussion

Physical properties of the extracts: The aqueous extract was prepared using maceration, whereas the ethanolic extract was prepared using soxhletation. The aqueous and ethanolic extracts of *Sesamum indicum* showed better yields. The aqueous extract which was blackish, showed a yield of 19.62% with semisolid texture. Whereas the ethanolic extract which was brownish black, showed a yield of 15.5% with oily texture. As the soxhlet apparatus is favourable for volatile solvents, water is not used here for the purpose of extraction. Hence, the aqueous extract was obtained from maceration process, but not by soxhelation. The ethanolic extract was dried completely at 80°C until a constant weight was obtained. As the boiling point of ethanol is 78.3°C, and here the extract was boiled at a temperature more than 78°C, there wouldn’t be any alcoholic residue in the extract. It confirms that the residual ethanol doesn’t interfere in the evaluation of antibacterial activity.

Phytochemical analysis: Preliminary phytochemical screening of aqueous and ethanolic extracts of *Sesamum indicum* was performed by using various phytochemical identification tests. These tests revealed the presence of carbohydrates, alkaloids, glycosides, saponins, flavonoids, polyphenols and tannins in both the aqueous and ethanolic extracts of *Sesamum indicum*. Whereas the ethanolic extract showed the presence of fixed oils and steroids in addition. The results of preliminary phytochemical screening of aqueous and ethanolic extracts of *Sesamum indicum* were tabulated in table – 1.

Antibacterial activity: The zone of inhibition of the seed extracts were compared with that of the zone of inhibition of standard drug. Both the aqueous and ethanolic extracts of *Sesamum indicum* showed better action against the Gram-negative bacteria (*Escherichia coli*) than that of the Gram-positive organisms (*Bacillus subtilis*). The standard drug tetracycline showed a zone of inhibition of 20 mm in case of Gram-positive organisms and 24 mm in case of Gram-negative organisms. As the doses of the extracts increased, an increase in the antibacterial activity was observed which was indicated by the increase in the zone of inhibitions of both the extracts. At a maximum dose of 300 µg/ml, the ethanolic extract of *Sesamum indicum* showed clear zone of inhibitions of 16 mm and 22 mm against the gram-positive and gram-negative organisms respectively. Whereas at the same dose, the aqueous extract of *Sesamum indicum* showed zone of inhibitions of 14 mm and 20 mm against the gram-positive and gram-negative organisms respectively. This clearly indicates that the ethanolic extract of *Sesamum indicum* showed better antibacterial activity than that of the aqueous extract. These results indicating the antibacterial activity of aqueous and ethanolic extracts of seeds of *Sesamum indicum* against *Bacillus subtilis* and *Escherichia coli* were

<table>
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<tr>
<th>Phytochemical Constituents</th>
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<tr>
<td></td>
<td>Aqueous Extract</td>
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<tr>
<td>Carbohydrates</td>
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<td>Fixed oils</td>
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<tr>
<td>Glycosides</td>
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<tr>
<td>Alkaloids</td>
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<td>Flavonoids</td>
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<td>Tannins</td>
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<td>Polyphenols</td>
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<td>Steroids</td>
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<td>Saponins</td>
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+ indicates presence; – indicates absence of the phytochemical constituents which were screened using various identification tests.
These results were thoughtful because in the present employed traditional method of treating a bacterial infection, preparing a plant extract with an organic solvent showed better antibacterial activity (14). In the whole study it was observed that the seed extracts showed high activity against a Gram-negative organism than that of a Gram-positive organism. The seeds of *Sesamum indicum* which were used for the study may contain the bio-components whose antibacterial potentials are highly comparable with that of the tetracycline against all Gram-negative and Gram-positive bacteria tested. The activity of the plant parts against both the Gram-negative and Gram-positive bacteria may be an indicative of the presence of broad spectrum antibiotic compounds in the plant (15). Many studies have supported this statement (16, 17, 18). The use of plants to heal diseases, including infectious one, has been extensively applied by the people (19). Today most of the pathogenic organisms are becoming resistant to antibiotics (20). To overcome this alarming problem, the discovery of novel active compounds against new targets is a matter of urgency. Thus *Sesamum indicum* could become a promising natural antibacterial agent with potential applications in pharmaceutical industry for controlling the pathogenic bacteria.

**Minimum inhibitory concentration:** The minimum inhibitory concentration values of both aqueous and ethanolic extracts were noted. The lowest MIC values were observed for aqueous extract at 88 – 91 µg/ml and for ethanolic extract at 92 – 95 µg/ml against the bacteria tested.

**Conclusion**

From the present study, it was concluded that both the aqueous and ethanolic extracts of seeds of *Sesamum indicum* possesses potent antibacterial activity which supports the traditional folklore. Further work will emphasize the isolation and characterization of active principles responsible for the antibacterial activity in clear and to establish the effectiveness and pharmacological rationale for the use of *Sesamum indicum* as an antibacterial agent.

**Acknowledgements**

The authors express their gratitude to Bapatla College of Pharmacy, Bapatla for providing the microbial culture samples. The authors are thankful to the management of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Guntur for providing the facilities to carry out the research work.
References


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