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Original Research Article

Antibacterial Activities of *Cassia angustifolia* Leaf Ethanolic Extract against Various Multiple Drug Resistant Microorganisms

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Abstract

Cassia angustifolia is a plant best known for its medicinal properties, commonly known as *Indian Senna*. This is a flowering plant belonging to the Fabaceae family. In this Study, the antibacterial activities of ethanolic extract of *Cassia angustifolia* leaves were evaluated against MDR Microorganisms. The antimicrobial activity of the extract was assessed against a range of bacterial strains, including *Escherichia coli* (MDR), *Klebsiella* sp. (MDR), *Pseudomonas* sp. (MDR), and *Acinetobacter* sp. (MDR). The result showed that the leaf extract of *Cassia angustifolia* exhibit significant antimicrobial activity against all tested MDR Microorganisms. The phytochemical content of the plant includes many bioactive compounds like *Tannins, Saponins, Flavonoids, Alkaloids, Steroids and quinones* etc which may be responsible for the observed antimicrobial activities. These findings suggest that *Indian Senna,* which could be used to develop new drugs for the treatment of resistant bacterial infections.

Keywords: *Cassia angustifolia;* Antibacterial activity; *Escherichia coli, Klebsiella* sp., *Pseudomonas* sp., *Acinetobacter* sp.

INTRODUCTION

Multidrug Resistance activity of bacteria is responsible for many critical infections posing a therapeutic challenge in the treatment of hospitalized as well as community based infected patients (Chatterjee *et al.,* 2016). For a long time in this civilization, plants play a crucial role as a source of natural products used for the health of human beings, and they are capable for producing various type of drugs. Senna (Latin name *Cassia angustifolia)* is a plant best known for its medicinal properties, commonly known as *Indian Senna*. *Indian Senna* is obtained from cultivated plants mainly in Asian country like south India and Pakistan. It was reported that the first variety of Senna was found along the *Nile River* in Egypt and Sudan. *Cassia angustifolia*, a native plant of Yemen, Somalia and now cultivated in other parts of the planet, features a sort of medical uses in Unani as well as other traditional system of medicine. The plant's leaves are the parts used for their medicinal benefits like Senna, contains *Anthraquinone compound*, particularly *sennosides* which stimulate the muscle of the intestine, promoting bowl movements (Laghari *et al.,* 2011). The dried leaves of *Cassia angustifolia* (Senna) have been used traditional Ayurvedic and Unani medicine for centuries as a remedy for constipation and other gastrointestinal disorder.

The active compound in Senna act as a natural laxative, increasing frequency and case of bowl movement. It is important to note *Cassia angustifolia* should be used with caution and under the guidance of healthcare professional as a prolonged use may lead to dependency and other side effect (Nascimento *et al.,* 2020). Constipation and Gastrointestinal disorder are treated by using leaf decoction. Leaves are the primary extract of *Cassia angustifolia* which are made in ethanol and evaluated the antimicrobial activity against many bacterial species. The effectiveness of these plant extract depends on both plant part and the solvent used for the extraction. All bacterial species were grown in Mueller Hinton Broth and for preparing the bacterial suspension two bacterial colonies of each plate were suspended in sterile 0.9% NaCl to obtain 10^8 CFU per mL as inoculums. Leaf extract's minimal inhibitory concentration (MIC) was determined against *Escherichia coli* (MDR), *Klebsiella* sp. (MDR), *Acinetobacter* sp. (MDR), *Pseudomonas* sp. (MDR).

Minimal inhibitory concentration is the lowest concentration of an antibiotic that inhibits the growth of a given strain of bacteria. It helps to evaluate the activity of new antimicrobial agents (Sasidharan *et al.*, 2011).

MATERIALS AND METHODS

Plant materials and Extraction

Cassia angustifolia which Sanskrit name is *Swarnapatri*, commonly known as *Indian Senna*, is a small perennial shrub that grows up to 1 meter in height, found throughout the year, cultivated largely in southern India, especially in Madurai, Tinnevally has also been introduced in Mysore. Mature, thick bluish colour leaves stripped off by hand, collected and dried in shade for 7-10 days till assume a yellowish green colour. The dried leaves (Fig.1) were collected in the month of June 2023. Then the dried leaves were packed separately in polythene bags and brought to the laboratory. The leaves were cut into small pieces by knife. Then ethanol extract of the sample was obtained by the following procedure. For extraction 1g of the leaves was kept in 5ml of ethanol for 48hr at room temperature. After that the extract was separated by centrifugationat 3000 rpm for 10 minutes (Sarkar *et al.*, 2023).

Figure 1: The leaf of *Cassia angustifolia* and Ethanolic extract

Test microorganisms and Culture Media

Four bacterial strains such as *Escherichia coli* (MDR), *Klebsiella* sp. (MDR), *Pseudomonas* sp. (MDR), *Acinetobacter* sp. (MDR) were selected for this study. *Escherichia coli* (MDR), *Klebsiella* sp. (MDR), *Pseudomonas* sp. (MDR), *Acinetobacter* sp. (MDR)are gram negative in nature. The bacterial species were grown in Mueller-Hinton broth.

Inoculum Preparation

From the stock cultures, each bacterial strain was streaked on agar plate. The plate was then incubated for 24 hours at 37°C. Then bacterial colonies emulsified in sterile 0.9% NaCl(w/v) to obtain 10^8 CFU per mL as inoculums for MIC value determination.

Antibacterial Screening assay (MIC)

The minimum Inhibitory concentration (MIC) assay was done by double serial dilution method, using 96 well plates. 100µL of Mueller-Hinton broth was dispensed in all the wells. Then 100µL extract concentration was added and mixed in the first well. Then the 100µL was added to the next well with 100µL Mueller-Hinton broth. Then the serial dilution was done till the eight well. Finally, 10µL of the different bacterial suspension was added to each well in separate rows. On the other hand, there was a control row chosen for each bacterial strains where the plant extract was not added. Only MH broth, bacterial suspension was present, and 100µL Ethanol was added and mixed it. Then the 100µL was added to the next well with 100µL Mueller-Hinton broth. Then the serial dilution was done till the eighth well. Then the optical density was measured at 620nm to be used as a baseline absorbance value. After measuring optical density, the plate was incubated 37°C for 24 hours. After 24 hours again optical density was measured at 620nm. Then the initial readings were subtracted from final readings. The MIC value determined the lowest concentration of the extract at which bacterial growth completely inhibited (Sultana *et al.*, 2019).

RESULTS

The analysis of result showed *Cassia angustifolia* extract was effective against all microbial strains tested in this study – MDR strains. *Escherichia coli* (MDR), *Klebsiella* sp. (MDR), *Acinetobacter* sp.

(MDR) all showed their MIC value of 0.78125 mg/ml. *Pseudomonas* sp. (MDR) showed a MIC value 1.5625mg/ml. All results are shown in Figures 2-5.

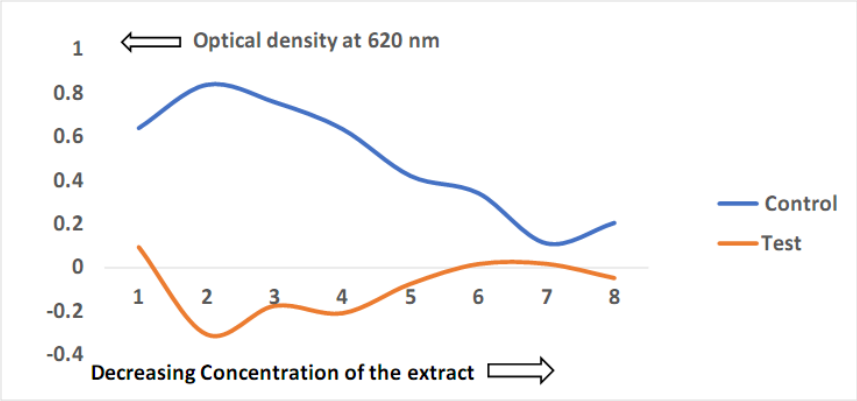

Figure 2: Showing antimicrobial activities of *Cassia angustifolia* leaf ethanolic extract against MDR *Escherichia coli.* Concentration of the extract: 1: 100mg/ml; 2: 50mg/ml; 3: 25mg/ml; 4: 12.5mg/ml; 5: 6.25mg/ml; 6: 3.125mg/ml; 7: 1.5625mg/ml; 8: 0.78125mg/ml

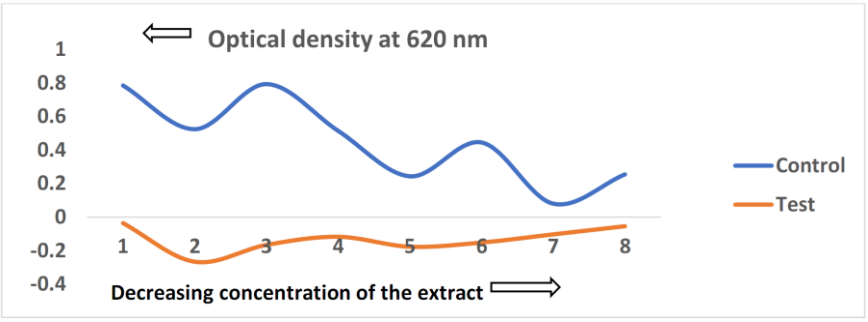

Figure 3: Showing antimicrobial activities of *Cassia angustifolia* leaf ethanolic extract against MDR *Klebsiella* sp. Concentration of the extract: 1: 100mg/ml; 2: 50mg/ml; 3: 25mg/ml; 4: 12.5mg/ml; 5: 6.25mg/ml; 6: 3.125mg/ml; 7: 1.5625mg/ml; 8: 0.78125mg/ml

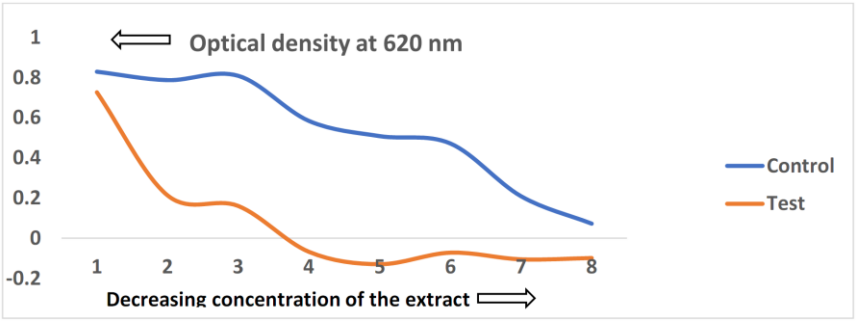

Figure 4: Showing antimicrobial activities of *Cassia angustifolia* leaf ethanolic extract against MDR *Acinetobacter* sp. Concentration of the extract: 1: 100mg/ml; 2: 50mg/ml; 3: 25mg/ml; 4: 12.5mg/ml; 5: 6.25mg/ml; 6: 3.125mg/ml; 7: 1.5625mg/ml; 8: 0.78125mg/ml

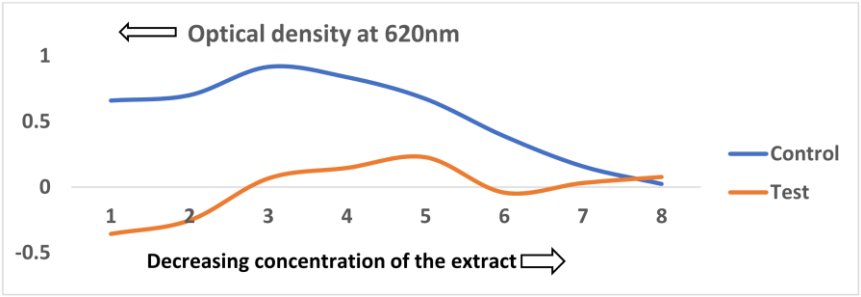

Figure 5: Showing antimicrobial activities of *Cassia angustifolia* leaf ethanolic extract against MDR *Pseudomonas* sp. Concentration of the extract: 1: 100mg/ml; 2: 50mg/ml; 3: 25mg/ml; 4: 12.5mg/ml; 5: 6.25mg/ml; 6: 3.125mg/ml; 7: 1.5625mg/ml; 8: 0.78125mg/ml

DISCUSSION

Healing with medicinal plants is as old as mankind itself (Petrovska *et al.*, 2012). The connection between man and his search for drugs in natures dates from the far past, of which there is evidence from various sources. Awareness of medicinal plant usage is a result of the many years of struggles against illness, pains due to which man learned to pursue drugs in seeds, leaves, fruit bodies and other plants of plant. Ever since ancient times in search for rescue for their disease, the people looked for drugs in nature. In view of the fact at the time there was not sufficient information either concerning the reasons for the illness which plant and how it could be utilized to curve everything was based on experience. In time the reasons for the usage of specific medicinal plants for treatment of certain diseases were being discovered thus the medicinal plant's usage gradually abandoned the empiric framework and became founded on explicatory facts. The findings of this investigation made it quite evident that *Cassia angustifolia* ethanolic leaf extract demonstrated antibacterial activity against tested pathogenic strains, including those that are resistant to antibiotics. The effectiveness of the active compounds present in leaf extract causes the inhibition of the growth of these tested pathogenic strains in the microtiter well.

The current findings revealed presence of bioactive compounds of flavonoid and anthraquinone type, in accordance to literature review. The lowest mic values of 0.78125mg/ml were recorded on *Escherichia coli* (MDR), *Klebsiella* sp. (MDR), *Acinetobacter* sp. (MDR), and the highest MIC value of 1.5625mg/ml was recorded on *Pseudomonas* sp. (MDR). The lower MIC value signifies that minimum amount of leaf extract is used to kill the bacterial species whereas a higher value signifies the use of comparatively more amount of sample for the control of any microorganism (Tatsimo *et al.*, 2017). Nearly, all the tests showed that extract showed some close inhibition against all tested bacterial strains, indicating that active ingredients in plant materials could be extracted into ethanol. The various components that are present in leaf extract of *Cassia angustifolia* are being separated using column chromatography in further research (Ahmed *et al.*, 2016).

CONCLUSION

In conclusion, the leaf extract of *Cassia angustifolia* has demonstrated potent antibacterial activity in various studies. The presence of secondary metabolites such as *Saponins, Flavonoids, Alkaloids, Steroids* in the extract is believed to be responsible for its antimicrobial activity. The extract has shown effectiveness against a range of pathogenic bacteria including multi drug resistant strains. The findings of these studies suggest that *Cassia angustifolia* could be a promising source of natural antibacterial agents for use in the developments of new drugs and therapeutic agents. The ethanolic extract of Indian senna had a similar antibacterial activity against gram negative bacteria. However, further research is needed to identify the specific compounds responsible for the extract's activity and evaluate their safety and efficacy in clinical settings.

Conflict of Interest

The author declares no conflict of interest.

Author's Contribution

Dr. Satadal Das conceived and designed the study and collected the plant sample. Mr. Sayantan Raha prepared the plant extract and carried out the experiment under Mr. Arup Kumar Dawn with the help of his proper guidance. Mr. Sayantan Raha analysed the data and wrote the manuscript. Dr. Satadal Das and others reviewed and edited the manuscript.

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