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

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Abstract **Original Research Article**

Cassia angustifolia (Indian Senna), a plant from the Fabaceae family, is an important medicinal plant of India and it is drought tolerant, hence it is cultivated under rain fed condition in marginal soils by small and marginal farmers of Gujarat, Rajasthan, Maharashtra, Andhra Pradesh and other Indian states. In this study, the antibacterial activities of ethanolic extract of *Cassia angustifolia* leaves were assessed against ATCC microorganisms - gram negative bacteria *Escherichia coli* (ATCC 25922) and gram positive bacteria *Staphylococcus aureus*(ATCC 25923). The result showed that *C. angustifolia* leaf extracts exhibited significant antimicrobial activity against both tested ATCC microorganisms. In this plant, *Resin, Phenol, Coumarins, Alkaloids, Saponin, Steroid*, etc, the phytochemical contents and the laxative principle *Sennoside* A and *Sennoside* B (two crystaline glucosides), which may be responsible for the observed antimicrobial activities. From these findings, we suggest that *C. angustifolia* (Indian senna) may be a possible source of natural antimicrobial agents, which could be used to develop new drugs for the treatment of resistant bacterial infections. Further studies on *C. angustifolia* on a large scale may open a new scope for other researchers to carry it.

Keywords: *Cassia angustifolia,*Antibacterial activity, *Escherichia coli, Staphylococcus aureus*.

1. INTRODUCTION

These days almost all countries of the world including India are facing a lot of problems in treating and curing infected patients worldwide. At this critical juncture, the study and research on *C. angustifolia* have become an essential step to the cause of the people across the world suffering from long standing health problems. Indian senna is a medicinal shrub that belongs to the Fabaceae family and is primarily grown in tropical region. Being a hardy species, it can be grown even in saline and rainfed conditions [1]. Cultivation of *C. angustifolia* does not require much expenses on irrigation, manuring, pesticidies, protection and other pre and post harvesting care. India is currently the world's primary source of cultivated Senna (with over 10,000 acres documented) [2]. Indian senna is grown commercially for its leaves and pods, purgatives that act mainly on the lower bowel. According to a laboratory study, the purgative ingredients are related to those of Aloes and Rhubarb, and the drugs properties are primarily due to *arthaquinone* derivatives and their glucosides. *C. angustifolia* is used for the treatment of various health issues. The leaves can also be ground into a paste and used to treat various skin conditions. The principal bioactive compound present in this leaves are *Sennosides*. The leaf extract may help in constipation due to the laxative property and induce diarrhea. The effectivity of *Cassia angustifolia* extracts from the plant leaves has been detected against many pathogens. Now in the various healthcare industry, ATCC bacteria is exponentially increased against many antibiotics [3]. Now it's time to develop newer antibacterial agents. The bacterial species were grown in Mueller Hinton Broth and for preparing the bacterial suspension three bacterial colonies from each plate were emulsified in sterile 0.9% NaCl to obtain 10^8 CFU per mL as inoculum.

2. MATERIALS AND METHODS

2.1 Plant Materials and Extraction

Recent years have seen a major spurt in the demand of medicinal plants not only with in the country but also for its expert. Senna (*Cassia angustifolia*) leaves and pods are commonly used as natural laxatives, both in the modern as well as in traditional system of medicines. India is also the largest producer

and exporter of this leaves, pods and total *sennosides* concentrate to the world market [4]. Conventionally, harvesting of Indian senna is done when the leaves are thick, bluish, and fully grown. Maximum yield of leaves is obtained under irrigated conditions of crops. *Cassia angustifolia* essential oil contains *a-pinene, b-pinene, b-caryophyllene, octanol, g-terpinolene, estragole, cis-limonene oxide, transanethole, caryophyllene oxide,* and *geranyl*. It contains the highest percentage of *cis-limonene oxide* and lowest amount of *a-pinene*. The dried leaves (Figure 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. The ethanol extract of the sample were obtained by the following procedure. For extraction 1g of the leaves was kept in 5ml of ethanol for 48hrs at room temperature. After that the extract was separated by centrifugation. The collected samples were centrifuged at 3,000 rpm for 10 minutes.

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

2.2 Test Microorganisms and Culture Media

Two bacterial strains such as the gram negative bacteria *Escherichia coli* (ATCC 25922) and the gram positive bacteria *Staphylococcus aureus* (ATCC 25923) were selected for this study. The bacterial species were grown in Mueller-Hinton Broth.

2.3 Inoculum Preparation

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

2.4 Antibacterial Screening assay (MIC value)

The Minimum Inhibitory Concentration (MIC) assay was done by double serial dilution method, using 96 well plates. 100 µ*L* of Mueller-Hinton broth was dispensed in 32 micro wells. Then 100µL extract concentration was added and mixed it 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 eighth 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 strain where the plant extract was not added. 100µL Ethanol was added and mixed it well. Then the 100µL was added to the next well with 100µL MH 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 absorbent value. After measuring optical density, the plate was incubated 37℃ for 24hrs. After 24hrs again optical density was measured at 620nm. Then the initial readings were substracted by final readings. The MIC value determined the lowest concentration of an antibiotic at which bacterial growth completely inhibited.

3. RESULTS

The analysis of result showed *Cassia angustifolia* extract was effective against all microbial strains tested in this study – ATCC strains. *Escherichia coli* (ATCC 25922) showed a MIC value 0.78125mg/ml and *Staphylococcus aureus* (ATCC 25923) showed a MIC value of 3.125mg/ml.

All results are shown in figure 2 and 3.

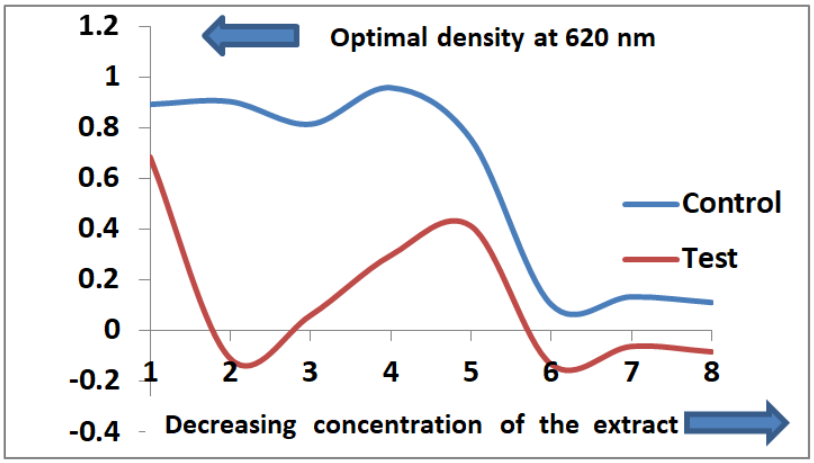

Figure 2: Showing antimicrobial activities of *Cassia angustifolia* leaf ethanolic extract against ATCC *Staphylococcusaureus*. Concentration of the extract: 1:100mg/ml; 2:50mg/ml; 3:25mg/ml; 4:12.5mg/ml; 5:6.25mg/ml; 6:3.125mg/ml; 7:1.5625mg/ml; 8:0.78125mg/ml

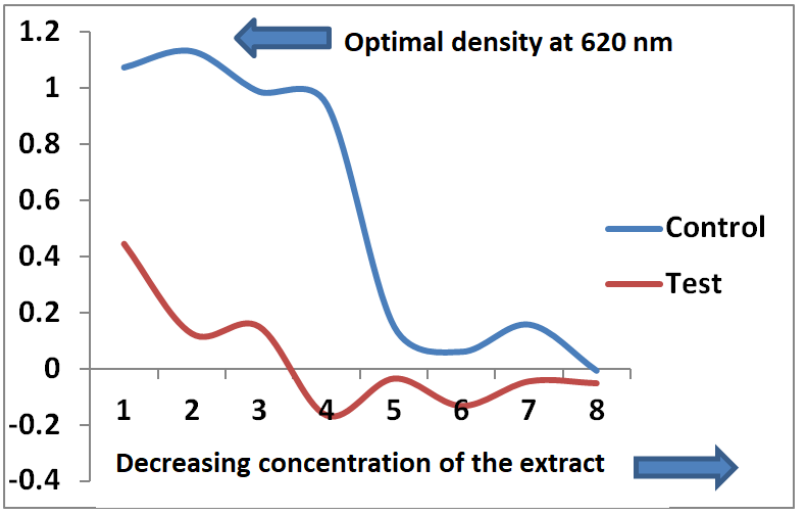

Figure 3: Showing antimicrobial activities of *Cassia angustifolia* leaf ethanolic extract against ATCC *Escherichiacoli*. Concentration of the extract: 1:100mg/ml; 2:50mg/ml; 3:25mg/ml; 4:12.5mg/ml; 5:6.25mg/ml; 6:3.125mg/ml; 7:1.5625mg/ml; 8:0.78125mg/ml

4. DISCUSSION

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. A medicinal plant contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [5]. Medicinal plants provide major source of molecules with medicinal properties due to presence of natural compounds. Medicinal plants are useful for curing human diseases and play an important role in healing due to presence of phytochemical constituents [6]. The findings of this investigation made it quite evident that Cassia angustifolia extracts demonstrated antibacterial activity against tested pathogenic strain, 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 isolation of bioactive compounds of *sennosides and anthraquinone* type, in accordance to literature review. The lowest MIC values of 0.78125mg/ml were recorded on *Escherichia coli* (ATCC 25922). And the highest MIC values of 3.125mg/ml were recorded on *Staphylococcus aureus* (ATCC 25923). The lower mic value signifies that minimum amount of leaf extract is used to kill the bacterial species where as a higher value signifies the use of comparatively more amount of sample for the control of any microorganism. Drugs with lower MIC scores are more effective antimicrobial agents 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.

5. 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, Resin, 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 ATCC 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 componds responsible for the extracts activity and evaluate their safety and efficacy in clinical settings.

5.1 Conflict of Interest: The author declares no conflict of interest.

5.2 Funding Source: This study was not supported any funding.

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6. Author's Contribution

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

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