

Antifungal Activities of *Cassia angustifolia* Leaf Ethanolic Extract against Various *Candida* Spp.

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Abstract

Original Research Article

Indian senna (*Cassia angustifolia*) is a perennial shrub belonging to the family Fabaceae. Indian senna contains flavonoids, pinnitols (polyols), acidic polysaccharides, and mineral substances. It also contains two types of glucosides known as sennosides A and B and other compounds, including myricyl alcohol, anthraquinone derivatives, galactose, arabinose, rhamnose, galacturonic acid, chrysophanic acid, salicylic acid, resin, and mannitol. The antifungal activity of the extract was assessed against fungal strains, including *Candida albicans* (ATCC10231), *Candida parapsilosis*, and *Candida auris*. The result showed that the leaf extract of *Cassia angustifolia* exhibits significant antifungal activity against all tested fungal strains. After the result, Indian Senna which could be used to develop new drugs for the treatment of resistant fungal infections. Further research and optimum preservation, and flavonoids extraction methods is needed, particularly in the developing world, where senna leaf and flower harvest processing methods are more traditional.

Keywords: *Cassia angustifolia*; Antifungal activity; *Candida albicans*; *Candida auris*; *Candida parapsilosis*.

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INTRODUCTION

The present investigation was aimed at evaluating the antifungal activity of ethanolic extract of *Cassia angustifolia*. It is a perennial shrub belonging to the family Fabaceae. It has been used traditionally by various civilizations across the world as laxative. The genus *Cassia* contains a range of 250 to 350 species. That are native to the tropical regions of Africa, Somalia, Sudan, Upper Egypt, Pakistan, and India. There is great variability in morphology, growth, habit, flower colour, height, and chemical constituents. Indian senna is a food and drug administration approved nonprescription medicine. Indian senna can cause some side effects including cramps, diarrhea, and stomach discomfort. Indian senna is possibly unsafe for long-term use by mouth or in high doses. Long-term use can also change the amount or balance of some chemicals in the blood (electrolytes) that can cause heart function disorders, muscle weakness, liver damage, and other harmful effects. It is the most important ingredient in colon-cleansing products that are used to repair the hemorrhoids. Senna contains sennosides that act on the bowel lining and cause the laxative affect. Hemorrhoids are caused by constipation. *Cassia angustifolia* is cross-pollinated by a variety of bees. There are a number

of plants outside of these genus with common names similar to Indian senna, including Alexandrian Senna, Cassia senna, and Easy Senna. *Cassia angustifolia* is known by different names depending on its origin. It is commonly known as Indian Senna in English, Shanaya and Hindi Sana in Hindi, Swarnpatri in Sanskrit, Nilavaka and Chinnukki in Malayalam, and Nelavrika and Sonamukkhi in Canada probably the most familiar Senna is Indian Senna that is *Cassia angustifolia* (Ahmed *et al.*, 2016). fungal resistance is also rising against various antibiotics due to the formation of biofilms by fungal strains (*Candida parapsilosis*, *Candida albicans*, etc). The fungal species were grown in Mueller Hinton Broth and for preparing the bacterial suspension. Leaf extract's minimal inhibitory concentration (MIC) is determined against *Candida albicans* (ATCC10231), *Candida parapsilosis*, and *Candida auris*. Fungal species were grown in Mueller Hinton Broth and for preparing the fungal suspension three colonies from each plate were emulsified in sterile 0.9%NaCl to obtain 10⁸CFU per ml as inoculums. *Candida parapsilosis* does not form true hyphae; it exists in either a yeast phase or pseudohyphal form. It is white, creamy, and shiny in dextrose agar and its cell shape is oval, round, or cylindrical. When *Candida parapsilosis* is in yeast form, its phenotype is smooth or cratered.

Candida auris due to its resistance to antifungal drugs and healthcare-associated outbreaks. Particularly concerning, is that *Candida auris* is multidrug resistant (i.e., non-susceptible to at least one agent in three or more classes of antifungal) and, in some cases, it has been found to be pandrug resistant (i.e., non-susceptible to all agents in all antifungal class (Nascimento *et al.*, 2020).

MATERIALS AND METHODS

Plant Materials and Extraction

Anthraquinone derivatives are the main active constituents of senna, which are responsible for its laxative properties. Indian senna has an aromatic odor due to the presence of essential oils in the leaves. The volatile oil of the leaves of *Cassia angustifolia* contains terpenes, aldehydes, and phenols. Besides the essential oil, the plant also contains salicylic acid, mannitol, saponin, resin, and sodium potassium tartrate (Laghari *et*

al., 2011). Isorhamnetin and kaempferol are also present in this plant. Other main chemical constitutions asitosterol, sennoside A, B (rhein and dianthrone), sennoside C, D (rhein, aloemodin, and heterodianthrone) and anthraquinones are present in free form. Small amounts of aloe-amine, rhein, chrysophanol, and their glycosides are detected in this plant. Harvesting of Indian senna is done when the leaves are thick, bluish, and fully grown. 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 were 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 centrifugation at 3000 rpm for 10 minutes (Sarkar *et al.*, 2023).



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

Test Micro Organisms and Culture Media

The three fungal strains such as *Candida albicans* (ATCC10231), *Candida parapsilosis*, and *Candida auris*. The fungal species were grown in Mueller-Hinton broth.

Inoculum Preparation

From the stock cultures, each fungal strain were streaked on agar plate. The plate was then incubated for 24 hours at 37°C. Then fungal 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 MuellerHinton 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 MuellerHinton 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 100 μ L Ethanol was added and mixed it. Then the 100 μ L was added to the next well with 100 μ L Mueller-Hintonbroth. Then the serial dilution was done till the eight 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 – fungal strains. *Candida auris*, *Candida parapsilosis* all showed their MIC value of 0.78125 mg/ml. *Candida albicans* showed a MIC value 1.5625mg/ml. The results are show in Figure 2 to 4.

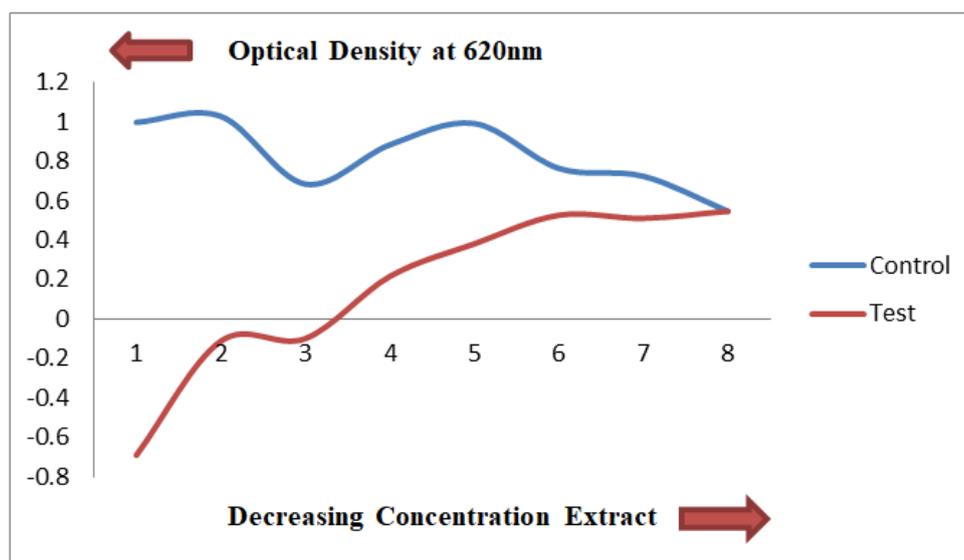


Figure 2: Showing antimicrobial activities of *Cassia angustifolia* leaf ethanolic extract against Fungus *Candida auris*, Concentration of the extract, 1: 100mg/ml; 2: 50mg/ml; 3: 25mg/ml; 4: 12.5mg/ml; 5: 6.25mg/ml; 6: 3.125mg/ml; 7: 1.5625mg/ml; 8: 0.78125mg/ml

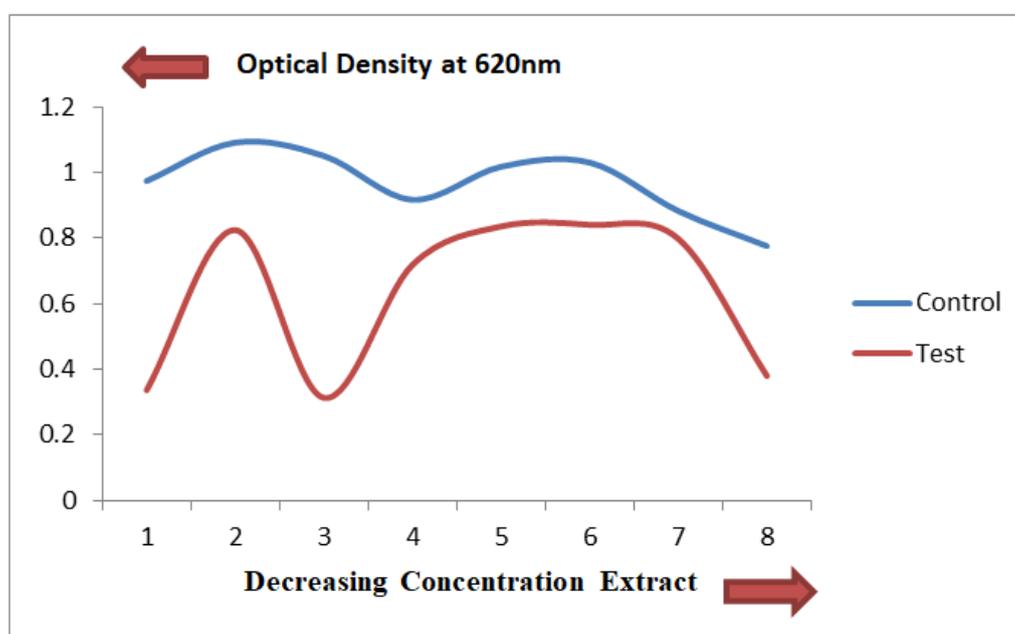


Figure 3: Showing antimicrobial activities of *Cassia angustifolia* leaf ethanolic extract against Fungus *Candida albicans* Concentration of the extract: 1: 100mg/ml; 2: 50mg/ml; 3: 25mg/ml; 4: 12.5mg/ml; 5: 6.25mg/ml; 6: 3.125mg/ml; 7: 1.5625mg/ml; 8: 0.78125mg/ml

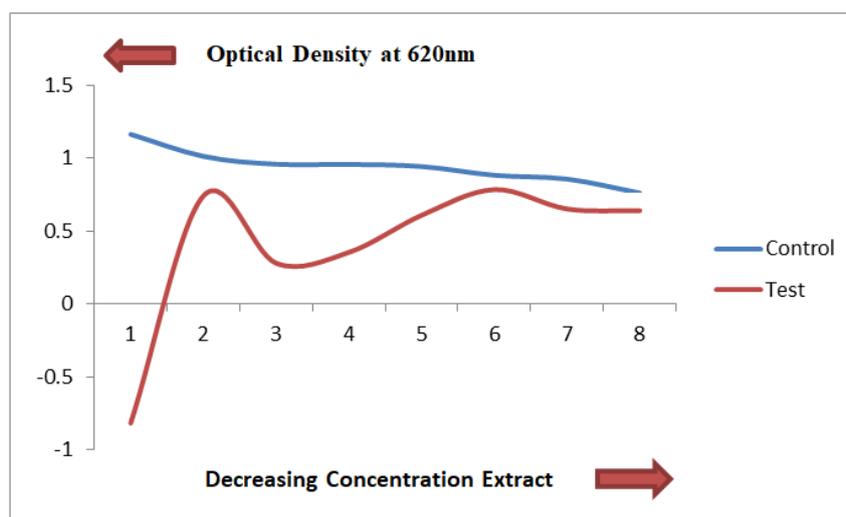


Figure 4: Showing antimicrobial activities of *Cassia angustifolia* leaf ethanolic extract against Fungus *Candida parapsilosis* Concentration of the extract: 1: 100mg/ml; 2: 50mg/ml; 3: 25mg/ml; 4: 12.5mg/ml; 5: 6.25mg/ml; 6: 3.125mg/ml; 7: 1.5625mg/ml; 8: 0.78125mg/ml

DISCUSSION

The medicinal value of these plants lies in some effective chemical compounds in different parts of the plant that produce a specific physiological effect in the human body (Petrovska *et al.*, 2012). Vegetable compounds are biologically active compounds that are naturally found in plants. Plant materials are often divided into primary and secondary receptors (Aggarwal *et al.*, 2011). Value-added products of *Cassia angustifolia* are flavored senna leaves, tea, sennosides, tablets, and other drugs. The products of Indian senna are available in various kinds of herbal formulations that are commonly used in pharmaceutical dosage in the form of tablets, capsules, powders, granules, liquids, pastes, and suppositories. Coated and uncoated tablets containing 40-60 mg of calcium sennoside contain 7.5e18 mg of hydroxyanthracene glycoside. Senna leaf powder is used as such and combined with some other powders such as cascara (bark from *Rhamnus purshiana*) and bgol (seed husks of psyllium, *Plantago ovate*). Senna paste is used for skin ailments. Granules and liquids are available in the form of syrup or fluid that are with or without alcohol. In the market, senna herbal teas and chocolates are also found. It can be used in a combination of herbs such as ginger, cloves, fennel, cinnamon, and coriander, although addition of other aromatics is designed for antinauseous effect. *Cassia angustifolia* is known to have strong antioxidant activity due to the presence of flavonoids. Essential oils have many biological and pharmaceutical properties such as antidiabetic, anticancer, antimutagenic, antiviral, anticancer, and antiinflammatory ones (Raut and Karuppaiyil, 2014). Antioxidants are the most important substances for the protection of bodily damage that is induced due to free radicals. The flavonoids can also have antimicrobial, antiinflammatory, antitumor, and other protective activity on human health. *Cassia angustifolia* ethanolic leaf extract demonstrated antifungal 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 isolation of bioactive compounds of flavonoid and anthraquinone type, in accordance to literature review. The lowest MIC values of 0.78125mg/ml were recorded on fungal strain - *Candida auris*, *Candida parapsilosis* and the highest MIC value of 1.5625mg/ml was recorded on fungal strain - *Candida albicans*. The lower MIC value signifies that minimum amount of leaf extract is used to kill the fungal 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 fungal 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.

CONCLUSION

In conclusion, the leaf extract of *Cassia angustifolia* has demonstrated potent antifungal 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 antifungal agents for use in the development of new drugs and therapeutic agents. However, further research is needed to identify the specific compounds responsible for the extract's activity and to 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. Ms. Megha Das Adhikary prepared the plant extract and carried out the experiment under Mr. Arup Kumar Dawn with the help of his proper guidance. Ms. Megha Das Adhikary analysed the data and wrote the manuscript. Dr. Satadal Das and others reviewed and edited the manuscript.

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