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IN- VITRO ANTIMICROBIAL ANALYSIS OF CASSIA ANGUSTIFOLA LEAVES EXTRACT AGAINST SOME COMMONE PATHOGENS

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Abstract

Cassia angustifolia, belongs to Fabaceae family. The genus cassia has traditional medicinal value to cure various illness caused by various pathogens. In the present work the results proved that Cassia angustifolia extracts possess a significant amount of secondary metabolites. Hydro-alcoholic extract is most effective solvent to extract the metabolites from the Cassia angustifolia. This also said that all the extracts possess detectable amount of phenols and flavonoids. The methanol extract possess high amount of phenols and flavonoids followed by water, acetone and hexane extract. The extracts also show antimicrobial activity against various pathogenic bacteria and fungi. Out of which methanol extract of Cassia angustifolia shows most effective antimicrobial activity followed by water, acetone and hexane extracts. Further work is needed to improve plant based drugs from Cassia angustifolia.

Key words: Cassia angustifolia, secondary metabolites, antimicrobial activity.

Introduction

Antibiotic resistance to a wide variety of human pathogenic bacterial and fungal strains has alarmingly increased in recent years, which is linked to the recurrence of infectious disorders. Antibiotic-resistant bacteria frequently cause infections in the respiratory system, circulation, and urinary tract worldwide [1]. Multidrug-resistant bacterial and fungal strains are the main cause of hospital acquired infections which reduce the efficacy of drugs and are ultimately responsible for treatment failure [2]. This situation has created a need to find more effective drugs. Natural products from microorganisms have been the primary source of antibiotics, and with the increasing acceptance of herbal medicines, the screening of medicinal plants for new active compounds has become a very important source of novel antibiotics [3].

Extensive researches on medicinal plants have also indicated that they are good sources of antioxidants [4–6]. They are involved in free radical scavenging activities which contribute to the protection from oxidative stress caused by the overproduction of free radicals and reactive oxygen species [7]. These are produced as by-products of various biochemical and physiological processes in the human body [8]. Bio molecular body systems such as lipids, DNA, RNA and proteins are adversely affected by the oxidative stress which eventually leads to human chronic diseases such as Alzheimer's, cardiovascular, atherosclerosis, cancer, stroke, fibrosis, aging and diabetes [9]. According to many research reports, the consumption of medicinal plants either in the form of raw extracts or chemical constituents is largely associated with lower risk of degenerative diseases caused by oxidative stress because they contain antioxidants such as phenolic, flavonoids, vitamins and carotenoids [10]. Phenolic compounds such as phenolic acids and flavonoids are reported to be involved in various biochemical activities like antioxidant, antimicrobial, antithrombotic, antiartherogenic, anti-

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inflammatory, anti-carcinogenic and antimutagenic [11]. Natural antioxidants of plant origin are usually more potent and beneficial than synthetic antioxidants such as propyl gallate (PG), butylated hydroxyl toluene (BHT), t-butyl hydroxyl toluene (TBH) and butylated hydroxyl anisole (BHA) [12]. It was also reported that synthetic antioxidants were the cause of carcinogenesis and liver damage in laboratory animals [13]. Thus, there is a need to explore and develop antioxidants of natural origin with greater efficacy and fewer side effects.

Cassia angustifolia L. (syn Cassia senna) in commerce is known as Indian or Tinnevelly Senna, is a well-known traditional medicinal plant belonging to family Caesalpiniaceae [14, 15]. It is a valuable plant drug in Ayurveda and modern system of medicine for the treatment of constipation [16, 17]. The plant available in market from different regions is likely to vary in quality and therapeutic activity due to difference in various geographical regions, difference in method of cultivation, collection, storage and seasons of plants collected [18]. The purpose of the present study is to compare the in vitro antimicrobial activity of aqueous and ethanolic leaf extracts of Cassia angustifolia L.

Materials and Methods

Preparation of Plant Materials Extraction: Cassia angustifolia leaves were collected from home farm during September 2022. The plant materials were weighed and were dried under sun light for 48 hours. Air dried coarsely powdered leaves were used for the antimicrobial screening. The Hydro-alcoholic, Ethanol, Methanol, Hexane, Chloroform and Petroleum ether extracts were prepared from each sample of Cassia angustifolia L. by extracting the 20g of each air dried coarsely powdered sample of leaves with 200 ml of 90% v/v alcohols and distilled water and different solvents respectively, the process of metabolite extraction were performed by using the soxhlet apparatus. The extracts were filtered through Whatman filter paper 42. The filterates were evaporated to dryness on the water bath. The prepared extracts were kept in desiccator and stored properly in refrigerator until required.

Antimicrobial Study

Antimicrobial activity is expressed as zone of inhibition in millimetres, which is measured with a zone reader. The Hydro-alcoholic, Ethanol, Methanol, Hexane, Chloroform and Petroleum ether extracts of Cassia angustifolia were screened for antimicrobial activity against a wide spectrum of microorganisms and the activity of extracts was compared with appropriate reference standards (Streptomycin for both Gram positive and Gram negative organisms and fluconazole for fungal strains). Microorganisms were grown in nutrient agar medium. Dimethyl Sulphoxide and distilled water were used as control.

Test organisms - The microorganisms used for the experiments were procured from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh.

Gram-positive organisms

Staphylococcus aureus (MTCC 3160)

Streptococcus mutans (MTCC 497)

Lactobacillus casei (MTCC 1423)

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Lactobacillus acidophilus (MTCC495)

Bacillus megaterium (MTCC 2187)

Gram-negative organisms

Enterococcus faecalis (MTCC439)

Escherichia coli (MTCC 1591)

Klabsiella pneumoniae (MTCC 2405)

Pseudomonas aeruginosa (MTCC 779)

Salmonella typhi (MTCC 531)

Fungal strains

Candida albicans (MTCC 10231)

Aspergillus niger (MTCC 1015)

Rhizopus oryzae (MTCC 262)

Candida rugosa (MTCC 96275)

All the plates were kept in a refrigerator at 2 to 8°C for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37 °C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. The solvent control was run simultaneously to assess the activity of Dimethyl Sulphoxide and water which were used as a vehicle. The experiments were performed three times. The diameter of the zone of inhibition was measured and recorded.

Results and Discussion

Antimicrobial Activity

Antibacterial Activity on gram positive strains

The antimicrobial activity of Cassia angustifolia leaves extracts were tested against five Gram positive bacteria Staphylococcus aureus, Streptococcus mutans, Lactobacillus casei, Lactobacillus acidophilus, and Bacillus megaterium. After proper incubation the results were recorded and represented in Fig.1. These recorded results said that hydro-alcoholic extract has high antimicrobial activity. These studies also proved that Bacillus megaterium was more sensitive to all the plant extracts followed by Lactobacillus acidophilus, Lactobacillus casei, Streptococcus mutans and Staphylococcus aureus. Antimicrobial activity of the above plant leaves extracts against gram positive organisms also proved that methanol is the most effective solvent for extracting broad spectrum of antimicrobial compounds from plant origin. Water and acetone extracts also showed the moderate antimicrobial activity, but hexane extract showed lesser activity. The inhibitory zones of different extracts varied with the type of microorganism involved in the work.

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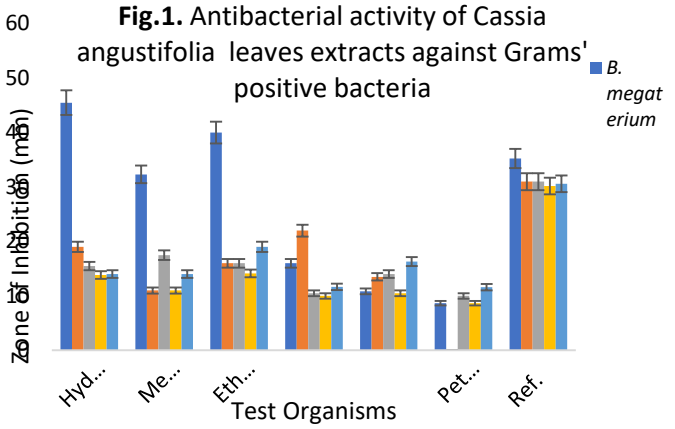


Fig.1. Antibacterial activity of Cassia angustifolia leaves extracts against Grams' positive bacteria

Antibacterial Activity on gram negative strains

Most of the pathogenic bacteria belong to Gram negative, they cause several diseases like sexually transmitted diseases, respiratory diseases, gastrointestinal problems, nosocomial infections etc. Owing to these problems, researches show more interest to isolate potential drugs against gram negative organism from plant origin. In the present study, four Gram negative pathogenic microorganisms were selected and tested against six extracts of Cassia angustifolia leaves. After proper incubation the diameter of inhibition zone was measured and results were recorded and represented in Fig: 2. Based on comparative analysis, the methanol extract of Cassia angustifolia showed better activity than the other extracts against above said gram negative organisms, followed by water, acetone and hexane extracts. These studies may helpful for the further isolation of antimicrobial drugs against gram negative organisms. The results of our research also highlight the fact that the methanol solvent extracts exhibited greater antimicrobial activity. So, the present observation suggests that the hydro-alcohol solvent extraction was suitable to verify the antimicrobial properties of medicinal plants which are also supported by many other investigators. [19,20,2,22]

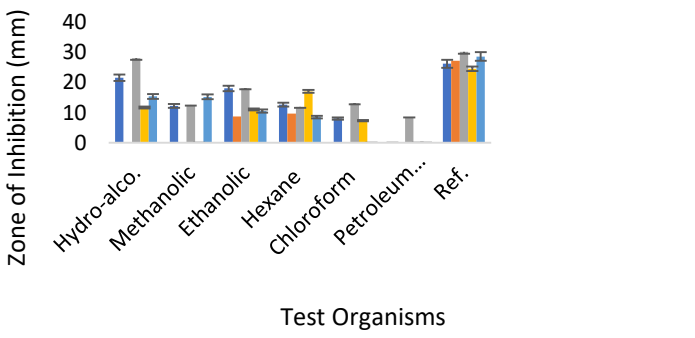


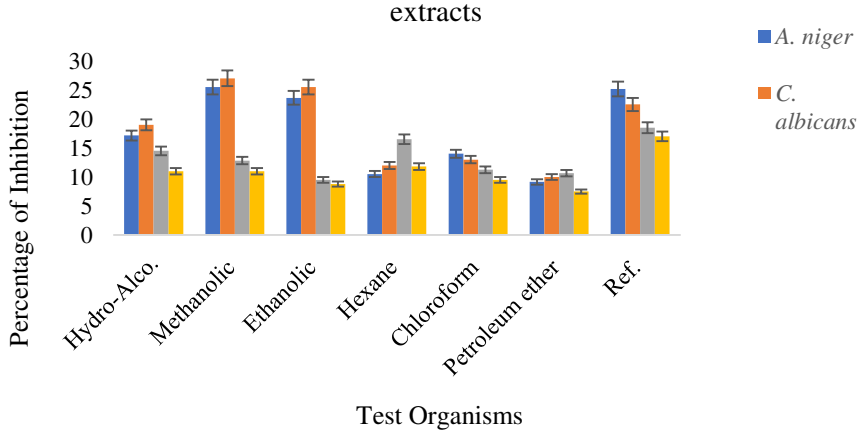
Fig.2. Antibacterial activity of *Cassia angustifolia* leaves extracts against Grams' negative bacteria

Anti-fungal Activity

In the present study all the extracts of Cassia angustifolia showed antifungal activity and the results were recorded and represented in Fig 3. From these results it was proved that methanolic extracts of above said plant showed highest antifungal activity, followed by ethanolic and hydro-alcoholic extract. Whereas Petroleum ether extract found lesser

antifungal activity than other extracts. Out of six extracts methanol extract found to possess highest antifungal activity against Candia albicans, Aspergillus niger, Rhizopus oryza and Candida rogasa. Out of four fungal species tested Candida albicans is more sensitive to Cassia angustifolia L extracts followed by Candia rogasa, Aspergillus niger, and Rhizopus oryza. The antifungal activity of these plants makes them potential source of antifungal agents and may be of economic importance as source of antifungal natural plant products. Our results are in partial agreement with those reported by Singh and Singh in 2000 and Natarajan et al. in 2005.

Fig.3. Antifungal activity of *Cassia angustifolia* leaves extracts



Conclusion

The present work concluded that Cassia angustifolia has significant secondary metabolites which are responsible for the various pharmacological activities. It also proved that a polar solvent, especially methanol is the most effective solvent to extract the metabolites from the Cassia angustifolia. The current results also said that all the extracts posses' detectable amount of phenols and flavonoids and antimicrobial activity against various pathogenic bacteria and fungi. The methanol extract of Cassia angustifolia has most effective antimicrobial activity followed by water, acetone and hexane extracts. Further work is needed to improve plant based drugs from Cassia angustifolia.

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