

ORIGINAL RESEARCH ARTICLE

Bioactivity of *Cassia auriculata* Methanol Extract against Human Pathogenic Bacteria and Fungi**P.K.Senthilkumar* and D.Reetha**

Department of Microbiology, Annamalai University, Annamalai Nagar, Chidambaram – 608 002, Tamil Nadu, India.

Received 15 Jul 2011; Revised 12 Oct 2011; Accepted 21 Oct 2011

ABSTRACT

Infectious disease can become a threat to public health in this world. The use of medicinal plants for the treatment of various diseases is an old practice in most countries and it still offers an enormous potential source of new anti-infective agents. The present study was conducted to evaluate the bioactivity of *Cassia auriculata* methanol extract against human pathogenic bacteria and fungi. The plant material was collected, shade dried and powdered. The powdered material was extracted using the organic solvent methanol. Antimicrobial activity of *Cassia auriculata* methanol extract was determined by Disc diffusion method. The zone of inhibition of *Cassia auriculata* methanol extract against bacteria was maximum against *Vibrio cholerae* followed by *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Escherichia coli*. The least zone of inhibition was recorded against *Salmonella typhi*. The Minimum Inhibitory Concentration (MIC) was ranged from 2mg/ml to 4mg/ml. The Minimum Bactericidal Concentration (MBC) value ranged between 2mg/ml and 4mg/ml. For fungi, the zone of inhibition was maximum against *Candida albicans*, followed by *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Candida tropicalis* and *Candida cruzei*. The least zone of inhibition was recorded against *Penicillium* sp. The MIC was 0.5mg/ml and the MFC value was 1mg/ml.

Keywords: *Cassia auriculata*, Methanol extract, Antimicrobial activity, MIC, MBC and MFC.

1. INTRODUCTION

Medicinal plants are a source of great economic value all over the world. Nature has been showed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. India is rich in all the 3 levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. In India, thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. Herbal medicine is still the main stay of about 75-80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents^[1]. Following the advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades advances in phytochemistry and in identification of plant compounds effective against certain diseases have renewed the interest in herbal medicines^[2].

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times^[3]. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry^[4].

India has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine. A country like India is very much suited for development of drugs from medicinal plant. Because of its vast and wide variations in soil and climate, the Indian sub –

continent is suitable for cultivation of large number of medicinal and aromatic plants which can be used as raw materials for pharmaceutical, perfumery, cosmetics, flavor, food and agrochemical industries. A large number of these plants grow wild and exploited especially for use in indigenous pharmaceutical houses. Some of these plants produce valuable drugs which have high export potential^[5].

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is found in "Rig Veda", which is said to have been written between 4500-1600 B.C. and is supposed to be the oldest repository of human knowledge. It is Ayurveda, the foundation of medicinal science of Hindu culture, in its eight divisions deals with specific properties of drugs and various aspects of science of life and the art of healing^[6]. Nowadays, there has been a revival of interest in herbal medicines. This is due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom. Plants are the basic source of knowledge of modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources. This burgeoning worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care.

Cassia auriculata (Tamil name – Aavaarai) is an erect herb found throughout the India in open areas of forest. It is widely distributed in poor soils, locally abundant in plains up to 900m. The leaves are bitter, astringent, acrid, thermogenic, haematinic, constipating and expectorant, seeds also bitter, astringent, acrid, cooling, opthalmic, diuretic alexeteric and vulnerary. The leaves are used for ulcer, leprosy and skin diseases, flowers are useful in diabetes and throat troubles. The leaf of this plant has been used in the traditional system of Indian medicine for the treatment of jaundice, liver disease, leprosy and ulcers. In this present study, the bioactivity of *Cassia auriculata* methanol extract was investigated against human pathogenic bacteria and fungi.

2. MATERIALS AND METHODS

2.1 Collection and drying of plant materials

The fresh and healthy leaves of *Cassia auriculata* were collected from in and around Chidambaram area, Tamil Nadu. The leaves of *Cassia auriculata* were washed thoroughly three times with water

and once with distilled water. The plant materials were shade dried and powdered. The powdered samples were hermetically sealed in separate polythene bags for further use.

2.2 Preparation of plant extract

The preparation of methanol extract of *Cassia auriculata* was done through modified method of Delgado and Navarro^[7].

2.3 Test microorganisms

The microbial isolates used in this present study were obtained from Rajah Muthaiya Medical College Hospital, Chidambaram. The bacterial cultures used in this study were *Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Vibrio cholerae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The fungal cultures used were *Candida albicans*, *Candida tropicalis*, *Candida cruzi*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Penicillium* sp.

2.5. Antimicrobial assays

2.5.1. Inoculum preparation

Twenty four hour old culture of selected bacterial/yeast culture was mixed with physiological saline and turbidity was adjusted by adding sterile physiological saline and turbidity was adjusted by adding sterile physiological saline until a McFarland standard of 0.5 (10^6 colony forming unit per ml) was obtained. The mould fungi were sub-cultured on Sabouraud Dextrose Agar and incubated at 35°C for 7 days. The growth was scraped aseptically, crushed and macerated thoroughly in sterile distilled water and the fungal suspension was standardized spectrophotometrically to an absorbance of 0.600 at 530nm.

2.5.2. Preparation of test solution and disc

The test solution was prepared with known weight of crude extracts dissolved in 5% Dimethyl Sulphoxide (DMSO). The sterile filter paper discs (6mm) were impregnated with 20µl of the *Cassia auriculata* methanol extract (corresponding to 100, 200 and 300mg/ml of *Cassia auriculata* methanol extract) and allowed to dry at room temperature.

2.5.3. Determination of Antibacterial activity and Antifungal activity by Disc diffusion method

The agar diffusion method was followed for antibacterial and antifungal susceptibility test. The Muller Hinton Agar and Sabouraud Dextrose Agar was prepared, poured on petriplates and allowed to solidify. After solidification, 0.1ml of standardized microbial inoculum suspension was poured and uniformly spread. The excess

inoculum was drained and the plates were allowed to dry for 5 minutes. After drying, the discs with *Cassia auriculata* methanol extract were placed on the surface of the plates with sterile forceps and gently pressed to ensure contact with the agar surface. Ciprofloxacin (5µg/disc) for bacteria and Amphotericin-B (100 units/disc) for fungi were used as the positive controls. The DMSO (5%) was used as a blind control. Finally the inoculated plates were incubated at 37°C for 24 hours (bacteria), 28°C for 24-48 hours (yeasts) and 28°C for 72-96 hours (mycelial fungi). The zone of inhibition was observed and measured in millimeters. Each assay in this experiment was repeated for four times.

2.5.4. Minimum Inhibitory Concentration (MIC)

The MIC of the *Cassia auriculata* methanol extract was tested in Muller Hinton Agar for bacteria, Yeast Nitrogen Base for Yeast and Sabouraud Dextrose Agar for mycelial fungi by Broth micro-dilution method. The extract was dissolved in 5% DMSO to obtain 128mg/ml. From the stock solution, 0.5ml of Muller Hinton Broth for bacteria, Yeast Nitrogen Base broth for yeast and Sabouraud Dextrose broth for mycelial fungi to get a concentration of 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 and 0.6mg/ml. 50µl of standardized suspension of the test organisms were transferred onto each tube. The control tube contained only organisms devoid of *Cassia auriculata* methanol extract. The culture tubes were incubated at 37°C for 24 hours (bacteria), 28°C for 48 hours (yeast) and 72 hours (mycelial fungi). The lowest concentrations, which did not show any other growth of tested microorganisms after macroscopic evaluation was determined as MIC.

2.5.5. Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The MBC and MFC of the *Cassia auriculata* methanol extract was determined by plating 100µl samples from each MIC assay tube with growth inhibition into freshly prepared Muller Hinton Broth for bacteria, Yeast Nitrogen Base broth for yeast and Sabouraud Dextrose broth for mycelial fungi and the plates were incubated at 37°C for 24 hours (bacteria), 28°C for 48 hours (yeast) and 72 hours (mycelial fungi). The MBC and MFC were recorded as the lowest concentration of the *Cassia auriculata* methanol extract that did not permit any visible any visible bacterial and fungal growth on the appropriate agar plate during the period of incubation.

3. RESULTS AND DISCUSSION

The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant although, it is usually not attributed to a single compound but a combination of the metabolites. The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct^[8]. The screening of plants usually involves several approach; ethno botanical approach is one of the common methods that are employed in choosing the plant for pharmacological study.

In the modern world multiple drug resistance has developed against many microbial infections due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants^[9].

In the present study, the antibacterial activity of the *Cassia auriculata* methanol extract was assayed against various bacterial pathogens and the results were showed in (**Table 1**). The zone of inhibition of 300mg of the *Cassia auriculata* methanol extract was maximum against *Vibrio cholerae* (21.2±0.8) followed by *Klebsiella pneumoniae* (19.0±1.0), *Staphylococcus aureus* (18.3±0.57), *Proteus mirabilis* (17.1±1.04), *Pseudomonas aeruginosa* (15.3±1.04) and *Escherichia coli* (13.01±0.76). The least zone of inhibition was recorded against *Salmonella typhi* (11.0±1.0). In comparison, the zone of inhibition of *Cassia auriculata* methanol extract against bacteria was more at 300mg/ml concentration when compared to other two concentrations (100mg/ml and 200mg/ml). For the positive control Ciprofloxacin, the zone of inhibition ranged from 28.3±3.21 to 33.01±2.64 mm. The Minimum Inhibitory Concentration was ranged from 2mg/ml to 4mg/ml. The Minimum Bactericidal Concentration value ranged between 2mg/ml and 4mg/ml.

Some studies concerning the effectiveness of extraction methods highlight that methanol extract

yields higher antibacterial activity than n-hexane and ethyl acetate (Sastry and Rao, 1994),^[10]. Whereas other report that chloroform is better than methanol and benzene (Febles *et al.*, 1995)^[11]. It is clear that using organic solvents provides a higher efficiency in extracting compounds for antimicrobial activities compared to water based method (Lima-Filo *et al.*, 2002)^[12]. Recently, Murugan and Saranraj (2011)^[13] investigated the antibacterial activity of *Acalypha indica* by Agar Well Diffusion Method. It was found that 50mg/ml of methanol extract of the plant able to inhibit the growth of nosocomial infection causing bacteria when compared to other solvent extracts. From this it was concluded that the solvent methanol able to leach out antimicrobial principle very effectively from the plant than the other solvents.

In this present research, the antifungal activity of the *Cassia auriculata* methanol extract was assayed against various fungal pathogens and the results were showed in (Table 2). The zone of inhibition of 300mg of the *Cassia auriculata* methanol extract was maximum against *Candida albicans* (23.3±1.15), followed by *Aspergillus fumigatus* (20.3±2.08), *Aspergillus flavus* (20.3±1.15), *Aspergillus niger* (20.3±0.57), *Candida tropicalis* (20.1±1.04) and *Candida cruzei* (19.3±1.52). The least zone of inhibition was recorded against *Penicillium* sp. (17.6±0.57). In comparison, the zone of inhibition of *Cassia auriculata* methanol extract against fungi was

more at 300mg/ml concentration when compared to other two concentrations (100mg/ml and 200mg/ml). For the positive control Amphotericin-B, the zone of inhibition ranged from 19.0±1.52 to 21.6±3.21 mm. The Minimum Inhibitory Concentration was 0.5mg/ml and the Minimum Fungicidal Concentration value was 1mg/ml.

Satish *et al.*, (2007)^[14] tested the aqueous extract of 52 plants from different families for their antifungal potential against eight important species of *Aspergillus* such as *Aspergillus candidus*, *Aspergillus columnaris*, *Aspergillus flavipes*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus ochraceus*, and *Aspergillus tamarii*. Among fifty-two plants tested, aqueous extract of *Acacia nilotica*, *Achras zapota*, *Datura stramonium*, *Embllica officinalis*, *Eucalyptus globules*, *Lawsonia inermis*, *Mimusops elengi*, *Peltophorum pterocarpum*, *Polyalthia longifolia*, *Prosopis juliflora*, *Punica granatum* and *Syngium cumini* have recorded significant antifungal activity against one or the other *Aspergillus* species tested. *Aspergillus flavus* recorded high susceptibility and hence solvent extracts viz., petroleum ether, benzene, chloroform, methanol and ethanol extracts of all the twelve plants were tested for their antifungal activity. In their results, they found that the methanol extract gave more effective than ethanol, chloroform, benzene and petroleum ether.

Table-1: Antibacterial activity of *Cassia auriculata* methanol extract

S No	Bacteria	Zone of inhibition (mm in dm)			Ciprofloxacin (5µg/disc)	MIC mg/ml	MBC mg/ml	Blind control (5% DMSO)
		100mg/ml	200 mg/ml	300 mg/ml				
1	<i>Escherichia coli</i>	10.8±0.76	11.1±0.76	13.1±3.51	31.6±3.51	4	8	No zone
2	<i>Salmonella typhi</i>	9.5±0.86	10.8±0.28	11.0±1.0	30.8±1.60	4	8	No zone
3	<i>Proteus mirabilis</i>	15.0±1.0	16.5±0.50	17.1±1.04	30.3±2.08	2	4	No zone
4	<i>Klebsiella pneumoniae</i>	18.0±0.76	19.0±1.52	19.0±1.0	29.6±3.05	2	4	No zone
5	<i>Vibrio cholerae</i>	16.6±0.56	18.5±0.50	21.6±0.28	33.01±2.64	4	8	No zone
6	<i>Pseudomonas aeruginosa</i>	13.0±1.0	13.4±0.57	15.3±1.04	28.3±3.21	4	8	No zone
7	<i>Staphylococcus aureus</i>	14.5±1.32	15.7±0.76	18.3±0.57	30.8±3.75	4	8	No zone

Table 2: Antifungal activity of *Cassia auriculata* methanol extract

S.No	Fungi	Zone of inhibition (mm in dm)			Amphotericin-B (100units/disc)	MIC mg/ml	MFC mg/ml	Blind control (5% DMSO)
		100mg/ml	200 mg/ml	300 mg/ml				
1	<i>Candida albicans</i>	19.6±1.52	21.8±1.75	23.3±1.15	23.0±2.64	0.5	1	No zone
2	<i>Candida tropicalis</i>	16.5±0.50	20.0±0.86	20.1±1.04	23.6±3.21	0.5	1	No zone
3	<i>Candida cruzei</i>	17.6±1.04	19.1±1.75	19.3±1.52	23.1±3.81	0.5	1	No zone
4	<i>Aspergillus niger</i>	19.3±1.52	19.6±1.52	20.3±0.57	21.0±3.0	0.5	1	No zone
5	<i>Aspergillus fumigatus</i>	17.8±1.04	17.5±1.32	20.3±2.08	22.6±3.78	0.5	1	No zone
6	<i>Aspergillus flavus</i>	17.8±0.76	18.6±0.57	20.3±1.15	23.6±3.05	0.5	1	No zone
7	<i>Penicillium</i> spp.	16.0±1.0	17.1±1.04	17.6±0.57	23.0±4.0	0.5	1	No zone

4. CONCLUSION

The study of antibacterial activity of herbal plant *Cassia auriculata* methanol extract showed

promising antibacterial activity against bacterial and fungal human pathogens. The results also indicated that scientific studies carried out on

medicinal plants having traditional claims of effectiveness might warrant fruitful results. These plants could serve as useful source of new antimicrobial agents.

REFERENCES

1. Akerele, O. 1993. Summary of WHO Guidelines for the Assessment of Herbal Medicines Herbal Gram. 22:13-28.
2. FAO, 1993. Medicinal and Aromatic plant in Asia. Bangkok, Thailand RAPA, Publication.
3. Farombi, E.O. 2003. African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. *African Journal of Biotechnology*, 2: 662-671.
4. Baker, J.T., R.P. Borris and B. Carte. 1995. Natural product drug discovery and development: New perspective on international collaboration. *Journal of Natural Products*, 58: 1325-1357.
5. Rastogi, R.P and B.N. Mehrotra. 2002. Glossary of Indian Medicinal Plants. National Institute of science communication, New Delhi, India.
6. Rathish R. Nair and Sumitra V. Chandra. 2005. *Punica granatum* - A potential source as antibacterial drug. *Asian journal of Microbiology, Biotechnology and Environmental Science*, 7: 625 - 628.
7. Delgado, G and Navarro, V. 1999. Two antimicrobial alkaloids from *Bocconia arborea*. *Journal of Ethanopharmacology*, 66: 223-226.
8. Parekh, J., D. Jadeja and S. Chanda. 2005. Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity. *Turkey Journal of Biology*, 29: 203-210.
9. Agrawal, P., V. Rai and R.B. Singh. 1996. Randomized, placebo controlled single-blind trial of holy basil leaves in patients with noninsulin-dependent Diabetes mellitus. *International Journal of Clinical Pharmacology and Therapeutics*, 34:406-409.
10. Sastry and Rao. 1994. Antibacterial substance from marine algae: Successive extraction using benzene, chloroform and methanol. *Bot. Mar.*, 37: 357-360.
11. Febles, C.L., Arias. A and Gil-Rodriguez MC. 1995. *In vitro* study of antimicrobial activity in algae (Chlorophyta, Phaeophyta and Rhodophyta) collected from the coast of Tenerife (in Spanish). *Anuria del Estudios Canarios* 34: 181-192.
12. Lima-Filo J.V.M., Carvola A.F.F.U and Freitas S.M. 2002. Antibacterial activity of extract of six macro algae from the North eastern Brazilian Coast. *Brazilian Journal of Microbiology*, 33: 311-313.
13. Murugan, T and P.Saranraj. 2011. Antibacterial activity of various solvent extracts of the Indian herbal plant *Acalypha indica* against human pathogens causing nosocomial infection. *International Journal of Pharmacology and Biological Archives*, 2 (5): 1498 – 1503.
14. Satish, S., D.C. Mohana, M.P. Ranhavendra and K.A. Raveesha. 2007. Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. *Journal of Agricultural Technology*, 3(1): 109-119.