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Antimicrobial Activity of Leaves and Flowers of *Cassia auriculata* linn

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Abstract

Since ancient times plant as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health. To treat chronic and infectious diseases plants used in traditional medicine contain a wide range of ingredients. In this regard, *Cassia auriculata* L. (Caesalpiniaceae) is widely used in Ayurvedic medicine as a tonic, astringent and as a remedy for diabetes, conjunctivitis, ulcers, leprosy, skin and liver diseases. The aim of present study was to evaluate the antimicrobial activity of ethanolic extract of *Cassia auriculata* leaves and flowers (CALE & CAFE). CALE and CAFE exhibited broad spectrum antimicrobial activity against standard strains of *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* and exhibited no antifungal activity against standard strains of *Candida albicans* and *Aspergillus niger*. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) was carried out for CALE and CAFE. The results obtained in the present study indicate that the CALE and CAFE can be a potential source of natural antimicrobial agents.

Key words: *Cassia auriculata,* Antimicrobial activity, Agar well diffusion method.

Introduction

For thousands of years nature has been a source of medicinal agents. WHO states more than 80% of the world's population relies on traditional medicine for their primary healthcare. There arises a need therefore to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies. In recent years, multiple drug/chemical resistance in both human and plant pathogenic micro organisms have been developed due to indiscriminate use of commercial antimicrobial drugs/chemical commonly used in the treatment of infectious diseases. In developing countries, people of villages and native communities use folk medicine for the treatment of common infections (Hassan *et al.,* 2009). This situation has forced scientists to search new antimicrobial substances in various sources (Kumar *et al.*, 2006). There are many approaches to search for new biologically active principles in higher plants. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Literature reports and ethnobotanical records suggest that the plants are the sleeping giants of pharmaceutical industry. It is expected that plant extract showing target sites other than the dose used by antibiotics will be active against drug resistant microbial pathogens and will provide novel or lead compounds that may be employed in controlling some infections globally (Ahmed *et al.,* 2001, Akinpelu *et al.*, 2006).

Cassia auriculata L. is one such herb, profoundly used in Ayurvedic medicine, known locally as 'avaram' and belonging to the family Caesalpiniaceae. *Cassia auriculata* is a shrub with smooth brown bark and is a common plant in Asia, India and Sri Lanka. The leaves are anthelmintic, good for ulcers, leprosy and skin diseases. The flowers are used in urinary discharges, diabetes and also for throat infection. The fruit is useful in thirst and in vomiting. The seed is used in diabetes, dysentery and chronic conjunctivitis. The bark is considered as astringent. The main objective of the present study was designed to investigate the antimicrobial efficacy of *Cassia auriculata* leaves and flower extracts (CALE & CAFE).

Material and Methods

Materials

Sabouraud dextrose agar (SDA), Mueller Hinton and Muller Hinton broth were purchased from Himedia laboratories Pvt. Ltd., Mumbai, while dimethylsulfoxide (DMSO) was pur-

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chased from Qualigens fine chemicals, Mumbai. Ofloxacin and flucanazole infusion were purchased from Cipla Pharmaceuticals, Mumbai. All other chemicals used in the study are of analytical grade purchased from respective suppliers.

Collection, preparation and extraction of plant material

The plant material consists of dried powdered leaves and flowers of *Cassia auriculata* belonging to the family Caeselpiniaceae. Fresh leaves and flowers of the plant were collected from Coimbatore district, Tamilnadu, India during the month of July, 2009. The plant was identified and authenticated by the Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India (BSI), Tamilnadu Agricultural University Campus, Coimbatore, bearing the reference number BSI/SC/5/23/09-10/Tech-255. The leaves and flowers of *Cassia auriculata* were collected, thoroughly dried under shade and powdered mechanically and sieved through No.20 mesh sieve. The finely powdered leaves and flowers were kept in an airtight container until the time of use.

The leaf extract was carried out by cold maceration and mechanical shaking method. The solvent used was 95% ethanol. About 60 g of powder was soaked with 600 ml of 95% ethanol for 12 h and then macerated at room temperature using a mechanical shaker for 4 h. The extract was filtered off and the marc was again soaked with the same volume of 95% ethanol for 12 h and then further extracted for 4h and filtered. The filtrates were then combined concentrated under reduced pressure and evaporated at 40°C. The percentage yield of the *C. auriculata* leaf extract (CALE) was 27.4% w/v.

The flower extract was carried out by continuous hot percolation method using Soxhlet apparatus. The solvent used was 95% ethanol. About 50 g of powder was extracted with 400 ml of solvent. The extract was concentrated to dryness under controlled temperature between 40-50ºC. The percentage yield of the *Cassia auriculata* flower extract (CAFE) was 18.2% w/v.

Phytochemical screening of the extract

Phytochemical screening were carried out for the ethanol extracts of leaf (CALE) and flower (CAFE) of *Cassia auriculata* for the presence of phytochemical constituents (alkaloids, flavonoids, glycosides, proteins, saponins, terpenoids, tannins and phenolics.

Antimicrobial tests

Test organism

Escherichia coli NCIM 2118; *Bacillus subtilis* NCIM 2010, *Staphylococcus aureus* NCIM 2127 were used to test antibacterial activity while *Candida albicans* NCIM 3100, *Aspergilus niger* NCIM 545 were used to assess antifungal activities. All the stock cultures were obtained from NCIM, Pune, India.

Preparation of inoculums

All organisms were grown over night (24 hr) at 37^{o}C on Nutrient Agar (NA) and harvested during the stationary growth phase. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to 10 ml of Mueller-Hinton (MH) broth for bacteria and Sabouraud dextrose (SD) broth for fungi that were incubated without agitation for 24 hrs at 37°C and 25^{o}C respectively. Bacterial inoculum was standardized by matching the turbidity of the culture to 0.5 McFarland standard by diluting with fresh MH broth and fungal inoculum was adjusted to the concentration of 10^{6}cCFU/ml by diluting with SB broth (Doughari, 2006).

Antimicrobial activity

The agar well diffusion assay was carried out to evaluate antimicrobial activity. Test organism was spread on corresponding agar plates (MH agar for bacteria and SD agar for fungi). The standard inoculums (NCIM cultures) were evenly spread on the surface of the medium. Wells of 6mm diameter were punched into the agar medium and filled with various concentrations of CALE and CAFE (0.5 to 4 mg/well) by dissolving in DMSO. Five wells were made, in each well different concentration of extract is added and the standard antibiotic (ofloxacin for bacteria and flucanazole for fungi) was filled in and this plate is kept in refrigerator for 20 minutes for diffusion. The plates were incubated for 24 hours at 37^{o}C for bacterial cultures, while the plates of fungal culture were incubated at room temperature (30-32^{o}C) for 48 h. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. The assay for CALE and CAFE against all micro organisms tested was performed in triplicates (Ahmad *et al.,* 1998, Jayaraman *et al.,* 2008, Doughari, 2006).

Determination of Minimum Inhibitory Concentration (MIC)

Serial 2-fold dilutions of the test antimicrobial agent were made in 1 ml of MH broth for bacterial and SD broth for fungal strain. Series of 10 to 15 dilutions of the test antimicrobial agents were prepared. Overnight cultures were grown at 37°C and diluted in MH broth and SD broth for fungal strain. This overnight culture was diluted to 10^5 CFU/ml. All tubes, including a control tube containing no drug, (representing positive control) were inoculated with one drop (0.05 ml) of standardized suspension of a strain (10^5 CFU/ml). The tubes were incubated at 37°C. Examined the tubes after 24 h and incubated further for 72 h, if necessary. A negative control was also kept. The determinations of MIC were performed in triplicate for each organism and the experiment was repeated where necessary. The MIC value for a given isolate were either identical, or within one dilution.

Determination of Minimum Bactericidal Concentration (MBC)

To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth were collected from those tubes which did not show any growth and inoculated on sterile MH agar (for bacteria) and SD agar (for fungi) by streaking. Nutrient agar and sabouraud agar only were streaked with the test organisms respectively to serve as control.

Plates inoculated with bacteria were then incubated at 37°C for 24 hours while those inoculated with fungi were incubated at room temperature (30 - 32°C) for 48 h. After incubation the concentration at which no visible growth was seen was noted as the minimum bactericidal concentration (Doughari, 2006).

Results

Phytochemical screening of the ethanolic extracts of CALE and CAFE revealed the presence of alkaloids, flavonoids, glycosides, proteins, saponins, tannins, phenols and terpenoids.

Antimicrobial activity

The results of antimicrobial assay of ethanolic extract of CALE and CAFE under study by agar well diffusion assay was shown in Table I. The antimicrobial activity was measured by zone of inhibition (mm). Totally three bacterial strains, two gram positive (*S. aureus, B. subtilis*) one gram negative bacteria (*E. coli*) and two fungal strains (*C. albicans* and *A. niger*) were used in this investigation.

Table I: Antimicrobial activity of CALE and CAFE on different bacterial and fungal species

Drug	Conc. mg/well	Zone of Inhibition (mm)				
		S. aureus	*B. subtilis*	*E. coli*	*C. albicans*	*A.niger*
CALE	0.5	-	10	12	-	-
	1	13	14	16	-	-
	2	16	17	18	-	-
	4	20	19	22	-	-
CAFE	0.5	-	-	-	-	-
	1	-	12	13	-	-
	2	11	13	14	-	-
	4	14	17	18	-	-
Ofloxacin	0.1	31	30	34	NA	NA
Flucanazole	0.05	NA	NA	NA	28	27

NA- Not applicable

The results of antimicrobial assay showed that the ethanolic extracts of CALE and CAFE were more active against gram negative bacteria (*E. coli*). On the contrary, the gram positive bacteria were more resistant (*S. aureus, B. subtilis*). CALE showed better growth of inhibition against *S. aureus, B. subtilis* and *E. coli* (20mm, 19mm, and 22mm) respectively at 4 mg. The CAFE generally showed low activity against test organisms (14mm, 17mm, 18mm at 4 mg conc.) compared to CALE. However, the activity of CALE and CAFE were less than the standard Ofloxacin. CALE and CAFE do not show any activity against fungal strains *C. albicans* and *A. niger.* The extract showed increasing inhibitory activity with increase in concentration (0.5 to 4 mg).

Determination of MIC

Results of minimum inhibitory concentration are shown in Table II. The MIC of CALE ranged between 128 to 512 µg/ml, with respect to all the test bacteria. The MIC of CAFE ranged between 1024 to 2048 µg/ml, with respect to all the test bacteria.

Table II: Minimum inhibitory concentration (MIC) values of CALE and CAFE on different bacterial strains in µg/ml

Bacterial strains	MIC(s) (µg/ml)	
	CALE	CAFE
S. aureus NCIM 2127	512	2048
B. subtilis NCIM 2010	256	1024
E. coli NCIM 2118	128	1024

Determination of MBC

Results of minimum bactericidal concentration are shown in Table III. The MBC of CALE ranged between 512 to 2048 µg/ml with respect to all the test bacteria. The MBC of CAFE ranged between 4096 to 8192 µg/ml with respect to all the test bacteria.

Table III: Minimum bactericidal concentration (MBC) values of CALE and CAFE on different bacterial strains µg/ml

Bacterial strains	MIC(s) (µg/ml)	
	CALE	CAFE
S. aureus NCIM 2127	2048	8192
B. subtilis NCIM 2010	2048	4096
E. coli NCIM 2118	512	4096

Discussion

Herbal medicines are a valuable and readily available resource for primary health care and complementary health care system. Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value that had to be discovered (Gislene *et al.,* 2000). In this aspect, *Cassia auriculata* (both CALE and CAFE) revealed for the presence of alkaloids, flavonoids, glycosides, proteins, saponins, tannins, phenols and terpenoids. Flavonoids have attracted a great deal of attention in relation to their potential for beneficial effects on health. Over the past few years, several experimental studies have demonstrated biological and pharmacological properties of many flavonoids, especially their antimicrobial activity (Narayana *et al.,* 2001), anti-inflammatory (Middleton *et al.,* 2000), antioxidant (Packer *et al.,* 1999; Robak, 1995) and anti-tumour (Castillo *et al.,* 1989; Inoue 1999) effects, which are associated with free radical scavenging actions (Geidam *et al.,* 2007). Flavonoids are found to be effective antimicrobial substance against a wide range of micro organisms, probably due to their ability to complex with extra cellular and soluble proteins and to complex with bacterial cell wall; more lipophilic flavonoids may disrupt microbial membrane (Natarajan *et al.,* 2005). Glycosides apart from exerting pronounced physiological effects are also known to have antiseptic properties (Robbinson, 1967). Saponins generally lower the surface tension and possess emulsifying activities, they tend to alter the permeability of the cell wall and hence exert a general toxicity on all organized tissues. They are known to possess some antibacterial activity. Tannin in the plant extract was found to possess antimicrobial activity (Basari *et al.,* 2005). Tannins are diverse organic substances with various compositions that have pronounced physiological astringent properties that hasten the healing of wounds and inflamed mucus membranes (Tyler *et al.,* 1988). Antimicrobial activity of tannins may be related to their ability to inactivate microbial adhesion enzymes and cell envelope transport proteins, they also complex with polysaccharides (Natarajan *et al.,* 2005). Many plant genetic resources have been analyzed for their active constituents possessing antimicrobial activities (Ates *et al.,* 2003). The demonstration of antimicrobial activity against both gram positive and gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds. This will be of immense advantage in fighting the menace of antibiotic refractive pathogens that are so prevalent in recent times. The antimicrobial activity of CALE and CAFE was evaluated by measuring the sizes of zones of growth inhibition produced by the extracts against the test organism as detailed in Table II. These inhibitions of growth of bacteria were as a result of the presence of the phytoconstituents identified in the crude extracts. The susceptibility of the test organism to the extract on the basis of growth inhibition zone diameters varied, according to micro organism, but generally, the largest inhibition zone diameters were recorded with the gram-negative bacteria and this is in agreement with the reports of El-Mahmood (2009). The inhibitory activity (measured by zone of inhibition) of CALE and CAFE was not pronounced against fungal strains *C. albicans* and *A. niger*. Many of the phytoconstituents with antibacterial properties in plants are preferably concentrated in leaves (Abubakar *et al.,* 2009). The demonstration of antimicrobial activity against both gram positive and gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds. The result also showed that the CALE are more effective than the CAFE. This may be due to the fact that the

leaves was more developed and mature than the flowers and may contain fewer pigments and other phenolics which have been reported to interfere with the antimicrobial activity of the extracts. The plant extracts tested did not show any anti-fungal (antimycotic) activity against any of the fungi at the tested concentrations.

Throughout this study, the solutions of the pure solvent (DMSO) was used as negative control and did not show any significant activity in form of growth inhibition against the test organisms. However the standard antibiotic Ofloxacin, though at lower concentration than the crude extracts, produced larger zones of growth inhibition than the crude extracts, i.e. Ofloxacin was more effective than the extracts as shown in Table II. Similar observations have been reported by other scholars (De, 2002; Kubmarawa *et al.,* 2002; El-Mahmood, 2007). Generally, antibiotics obtained from micro organisms and or the synthetic processes are more effective at lower doses that plant based products. The quantity of active ingredients of plant origin required to cause inhibition of growth may not matter since medicinal plants have been reported to have little or no side effects (Hassain 2002).The scientific literature is full of reports of bioactive compounds isolated from plants having antimicrobial properties, but none of these plant based chemicals have been successfully exploited for clinical uses as antibiotics (Gibbons, 2004).

The MIC is a helpful parameter used to assess bacteriostatic activity of antibacterial agents while the MBC is used to detect bactericidal activity under similar conditions. The MBC values are generally more reliable than the MIC values (Junaid *et al.,* 2006). This study showed that the highest MIC and MBC values of *S. aureus* is an indication that either the plant extracts are less effective on some gram positive bacteria or that the organism has the potential of developing antibiotic resistance, while the low MIC and MBC values for other bacteria is an indication of the efficacy of the plant extracts (Dougari, 2006). The MIC values can either be the same or lower than the MBC values (Croshaw, 1983). The MIC and MBC values shown in Table IV and Table V reveals the effectiveness of the extracts and this is very important for people who depend on this plant for their health care needs (Abubakar, 2009).

These observations may be attributed to the nature of biologically active components (Alkaloids, saponins and tannins) which could be enhanced in presence of ethanol. It has been documented that these components are well known for antimicrobial activity. In addition to that, the stronger extraction capacity of ethanol could have produced greater the active constituents responsible for antimicrobial activity.

Conclusion

This study has provided a scientific basis on the use of *Cassia auriculata* in herbal medicine and can be used as antimicrobial plant in the development of new drugs for the treatment of infectious disease.

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