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In vitro antimicrobial activity and phytochemical analysis of *Cassia auriculata* Linn

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ABSTRACT

This study was performed to evaluate the antimicrobial activity of aerial parts of chloroform extract of *Cassia auriculata L.* The chloroform extract of *C. auriculata* were shown to possess an antimicrobial activity against two gram positive and two gram negative human pathogenic bacteria and fungi, *viz. Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and fungus cultures *Candida albicans* and *Aspergillus niger* by using disc diffusion method. The extract showed antibacterial activity at all concentrations selected, but only the extract with the concentration of 300µg/ml showed maximum antibacterial activity against all the organisms except *Pseudomonas aeruginosa* which are comparable with the standard control, amikacin. The anti fungal activity of chloroform extract of *C. auriculata* revealed significant effect against *Candida albicans* and *Aspergillus niger* with the net inhibition zone of 14 and 14 mm, respectively at 300µg/ml concentration, which is almost comparable with standard control, ketokonazole used as an antifungal agent. The phytochemical analysis showed the presence of alkaloids, carbohydrates, fixed oils, fats, tannins, gum & mucilage, flavonoids, saponins, terpenoids, lignin and sterols. It is concluded that the antimicrobial activity showed by the plant was due to the presence of these phytochemicals. Further studies are highly needed for future drug development.

***Key Words*:** Disc diffusion, amikacin, ketokonazole, chloroform extract, soxhlet extractor, pathogenic bacteria.

INTRODUCTION

Herbal medicines have been known to man for centuries and they have frequently used plants to treat common infectious diseases, and some of these traditional medicines. The therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine (Nayan *et al.*, 2011, Dogruoz *et al.*, 2008). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine. Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional systems of medicine (Sukanya *et al.*, 2009).

Antibiotics are one of the most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains (Dogruozet *et al.*, 2008). Increasing development of drug resistance in human pathogens as well as the appearance of side effect of synthetic drugs need to developed new antimicrobial drugs from natural sources. This situation has forced to search new antimicrobial substances in various sources like medicinal plants (Doshi *et al.*, 2011; Tomoko *et al.*, 2000). The Medicinal plants are considerably useful and economically essential and it contain rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties (Khan *et al.*, 2009). The use of plant extracts and phytochemical both with known antimicrobial properties are of great significance. In the past few years, a number of investigations have been conducted worldwide to prove antimicrobial activities from medicinal plants. A number of phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases due to their availability, fewer side effects and reduced toxicity.

Cassia auriculata commonly known as *Tanners Cassia*, also known as "Avaram" in Tamil is a shrub that belongs to the *Caesalpiniaceae* family (Thulasi and Amsavenit, 2012) is of great importance to tanner and workers in iron as well known for its contribution in Ayurveda as Avarai Panchaga Choornam and Kalpa Herbal tea. The root of the plant is used in decoction as alternative as well as medicinal oil prepared from the bark in Tamil called as *averai – yennai*. The leaves infused yield a cooling drink and ground to paste with water and the seeds of *Phaseolus radiatus* and poppy seed they are applied to herpetic eruptions. The Flowers of the plant are used in preparation of tea, which is prescribed in diabetes. Compound syrup is prepared with the flowers, mocharas and Indian saparilla which are prescribed for nocturnal emissions. The seeds are used in diabetes, opthalamia and chylous urine (Doshi *et al.*, 2011). Every part of the plant is valuable in medicine for ulcers, leprosy and liver disease. The plant can also be used as an antidiabetic, hypolipidemic and anti-oxidant. According to Ayurveda, the different parts of plant have been used for various ailments. Roots are useful in urinary discharges and cures tumors, skin diseases and asthma. Powder of bark is used for fixing teeth and decoction for chronic dysentery. Decorticated seeds in fine powder and paste are valued local applications to purulent opthalamia and conjunctivitis (Tomokoet *et al.*, 2000). In the present investigation an attempt has been made to enrich the knowledge of antimicrobial activity of chloroform extract of the aerial parts of *C. auriculata* L.

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MATERIALS AND METHODS

Collection and Drying of plant materials

Healthy aerial parts of the *C. auriculata* (stem, leaves, flowers and seeds) were collected from the Herbal garden, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu. The collected plant was authenticated by the Head, Department of Botany, Annamalai University, Annamalai nagar, Tamil Nadu and a voucher specimen (No. 1958) was kept in the Pharmacognosy Lab, Department of Pharmacy, Annamalai University for future reference. The plant was washed thoroughly three times with purified water and once with distilled water. The plant materials were air shade dried and then powdered using electric blender to get a coarse powder. The powdered samples were kept in sealed containers for extraction purposes.

Collection of Microorganism

The microorganisms used in this experiment were *Bacillus subtilis* (10876), *Staphylococcus aureus* (29837), *Pseudomonas aeruginosa* (27853), *Escherichia coli* (1129) and fungus culture *Candida albicans* and *Aspergillus niger*. They were obtained from Boss Laboratories, Madurai, India.

Preparation of plant extract

The air-dried and powdered plant material 50 g was extracted successively with 500 ml of petroleum ether, chloroform, ethyl acetate and methanol by using a soxhlet extractor until a complete extract were effected (10-12h) at a temperature not exceeding the boiling point. The extracts were evaporated to dryness under reduced pressure using a Rota vapor (Buchi Flawil, Switzer-land) and the resulting pasty form extracts were stored in a refrigerator at 4°C for Phytochemical screening (Shankara *et al.*, 2012).

Preliminary Phytochemical screening

The extracts were subjected to preliminary Phytochemical testing to detect for the presence of different chemical groups of compounds. The plant extracts was carried out qualitatively for the presence of Alkaloids, carbohydrates, fixed oils, fats, tannins, gum & mucilage, flavonoids, saponins, terpenoids, lignin and sterols by using the standard method given by (Harborne, 1998).

Antimicrobial screening

The antimicrobial activity of the *C. auriculata* extracts was determined by using disc diffusion method. Two gram positive bacteria and two gram negative bacteria were used for this study. The organisms were sub-cultured on Mueller Hinton Agar medium, incubated at 37°C for 24 h and stored at 4°C in the refrigerator to maintain stock culture. Petri plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA) (HIMEDIA, Mumbai, India). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. The tests were conducted at three different concentrations at 100,200 and 300 µg /ml respectively of the crude extract. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Amikacin (50µg/ml) was used as positive control. The plates were incubated for 24 h at 37°C. The diameter of the zone of inhibitions was measured by measuring scale in millimeter (mm) (Sharmeen *et al.* 2012). The sensitivity of the microorganisms to plant extract was determined by measuring the size of inhibitory zones on the agar surface around the discs (kainsa *et al.*, 2012, Saranraj *et al.*, 2010).

Table 1: Phytochemical investigation of Aerial parts of the *C. auriculata* Linn.

Sl. No.	Constituents	Pet. Ether	Chloro-form	Ethyl acetate	Meth-anol
1	Alkaloids	-	+	-	+
2	Carbohydrate	-	-	-	+
3	Fixed oil & fats	+	-	-	-
4	a. Tannins	-	+	+	-
	b. Phenols	-	+	+	-
5	Gum & Mucilage	+	-	-	-
6	Flavonoids	-	+	+	+
7	Saponins	-	-	+	+
8	Terpenoids	-	-	-	-
9	Lignin	-	-	-	-
10	Sterols	+	-	-	-

Anti fungal screening

Fungus culture *Candida albicans* and *Aspergillus niger* were used for this study. The anti fungal activity was performed according to the standard reference method (Subramanion *et al.*, 2010). The extracts were dissolved in 2% DMSO. The initial concentration of extract was 100µg/ml. The initial test concentration was serially diluted twofold. Each well was inoculated with 50 µg/ml of suspension containing 104 spore/ml of fungi. The anti fungal agent ketokonazole was included in the assays as positive control. The plates were incubated between 24 h and 72 h at 27°C. The sensitivities of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks (Rehman *et al.*, 2002).

RESULTS AND DISCUSSION

The use of antimicrobials has increased steadily since the discovery of penicillin. Numerous drugs have been developed since then, few of which were considered potentially toxic. A number of factors contribute to antibiotic resistance including misuse and overuse of antibiotics in humans, animals and agriculture; patient's demand for and receipt of antibiotics when they don't need them; and failure to finish an antibiotic prescription. Therefore the use of Ayurveda medicines has increased now days (Senthilkumar and Reetha, 2011).

The bio active compounds obtained from medicinal plants have been used to treat various ailments casued by microorganisms. The most important of these bioactive principles are alkaloids, phenolic compounds, flavanoids and tannins that may be evolved in plants as self defence against pests and pathogens (Sukumaran *et al.*, 2011). The Extractive values of aerial parts of C. *auriculata* Linn using different solvent showed petroleum ether 0.50, chloroform 1.20, ethyl acetate 2.15, methanol 2.56. It was found that chloroform extract aerial parts of the *C. auriculata* Linn contained Alkaloids, carbohydrates, fixed oils, fats, tannins, gum & mucilage, flavonoids, saponins, terpenoids, lignin and sterols when compared with other three extracts *viz*,. petroleum ether, ethyl acetate and methanol. The results (table 1) showed that Chloroform was the best solvent for extracting the effective antimicrobial substances from the medicinal plant *C. auriculata* than the other three solvents. Therefore, the chloroform extract has been selected for investigating antimicrobial activity. The antibacterial activity of *C. auriculata* suggests that the extract contains the effective active Phytochemical responsible for the elimination of microorganisms.

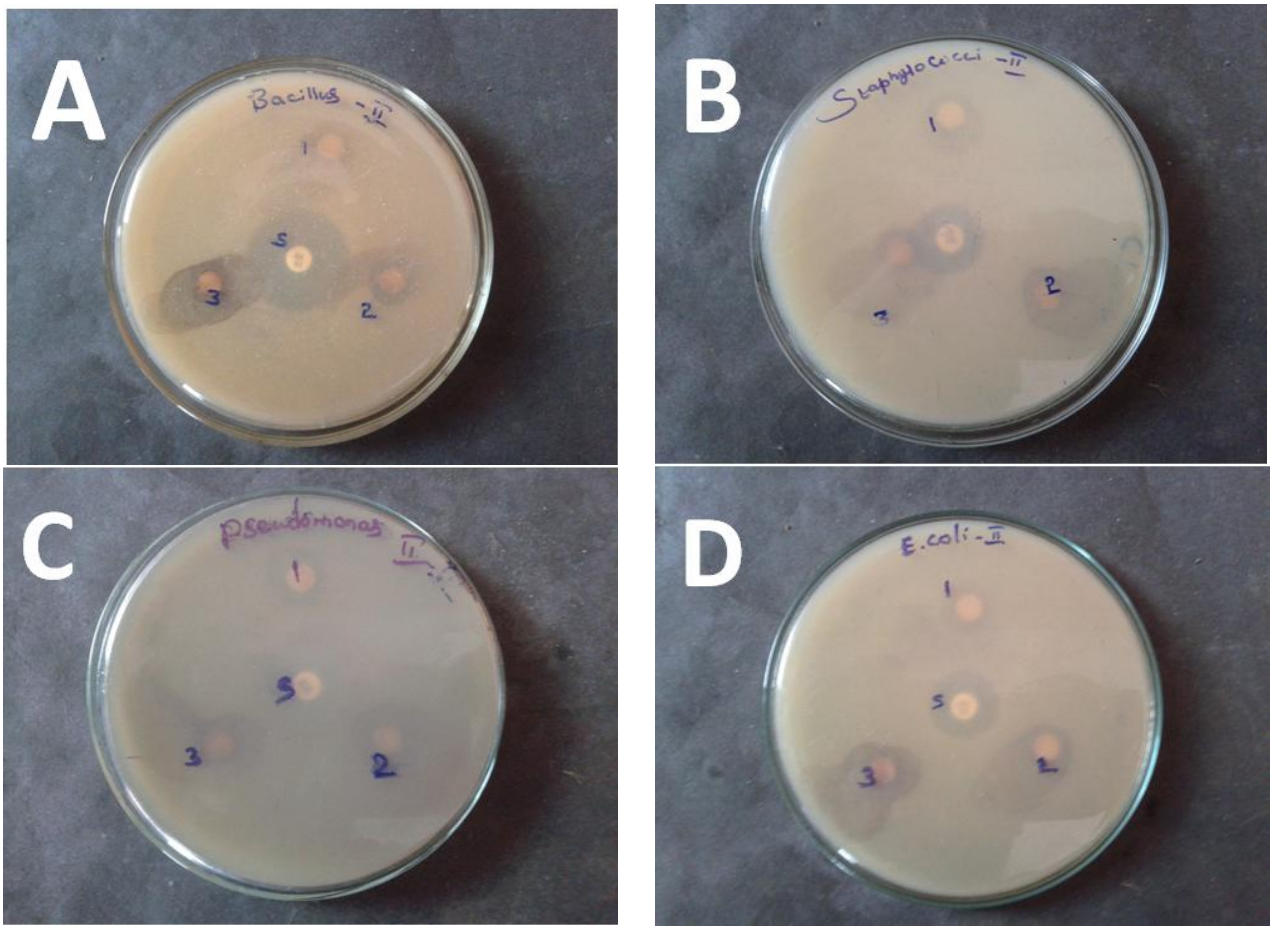

Figure 1: Inhibition of bacterial growth by chloroform extract of *C. auriculata* by Disc diffusion method.

A- *Bacillus subtilis*, B-*Staphylococcus aureus*, C-*Pseudomonas aeruginosa*, D- *Escherichia coli*

'1','2' and '3' represents zone of inhibition of chloroform extract of *C.auriculata* at the concentration of 100, 200 and 300 µg/ml, respectively. The centre zone 'S' represents zone of inhibition of standard antibacterial agent (Amikacin) at the concentration of 50 µg/ml.

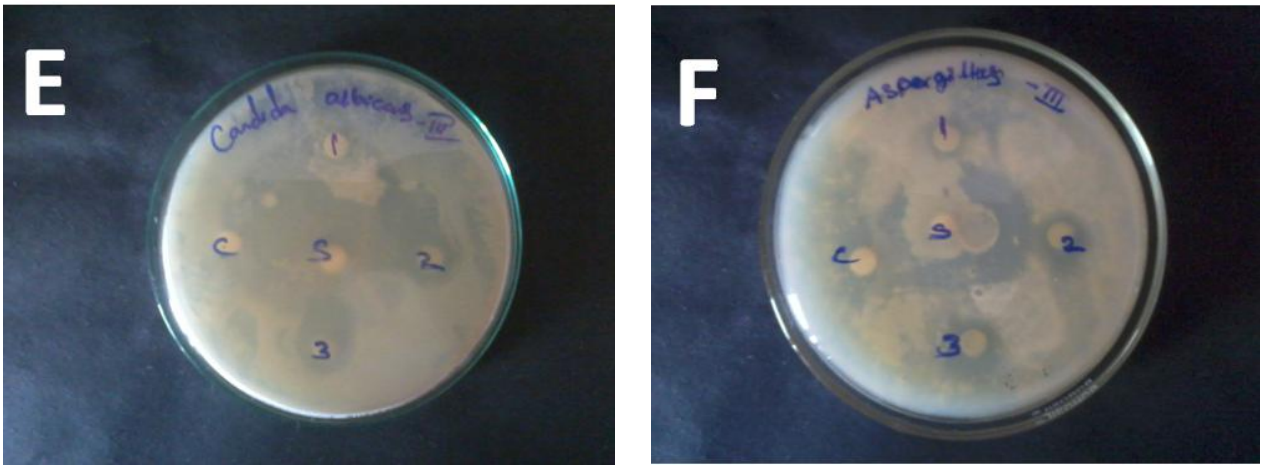

Figure 2: Inhibition of fungal growth by chloroform extract of *C. auriculata* by disc diffusion method.

E-*Candida albicans*, F-*Aspergillus niger*

'1','2' and '3' represents zone of inhibition of chloroform extract of *C. auriculata* at the concentration of 100, 200 and 300 µg/ml, respectively. The centre zone 'S' represents zone of inhibition of standard antifungal agent (ketokonazole) at the concentration of 50 µg/ml and 'C represents zone of control.

Table 2: Antibacterial activity of *Cassia auriculata* Linn in different strains

Drug	Conc. (µg/ml)	Zone of inhibition			
		B. subtillis	*S. aureus*	*P. aeruginosa*	*E. coli*
Cassia auriculata	100	9	4	NI	7
	200	11	7	NI	8
	300	15	12	NI	12
Standard	50	20	18	17	18

NI – No Inhibition

Table: 3. Anti fungal activity of *Cassia auriculata* extract in different strains

Drug	Conc. (µg/ml)	Zone of inhibition	
		Candida albicans	*Aspergillus niger*
Cassia auriculata	100	8	10
	200	12	13
	300	14	14
Control	-	NI	NI
Standard	50	16	17

NI – No Inhibition

The *in vitro* antibacterial activities of chloroform extract of *C. auriculata* were found to have maximum activity against all organisms except *Pseudomonas aeruginosa*. The extract showed (table 2, figure 1) antibacterial activity at all concentrations selected, but only the extract with the concentration of 300µg/ml showed maximum antibacterial activity against the organisms which are comparable with the standard control, Amikacin. The anti fungal activity of chloroform extract of *Acalypha indica* against *Candida albicans* and *Aspergillus niger* by using disc diffusion method revealed significant effect against the above two organisms with the net inhibition zone of 14 and 14 mm, respectively at 300µg/ml concentration, which is almost comparable with standard control, ketokonazole, an antifungal agent (table 3, figure 2). This study compares the antimicrobial properties obtained by a plant and which is easily available to the common man. It may have fewer side effects as it falls in the category of natural medicine. The present study exhibited the antimicrobial effect of Chloroform extract justified the medicinal use of *C.auriculata* and further study is required to find out the active component of medicinal value.

CONCLUSION

It is concluded based on the findings of the present study that the aerial parts of *C. auriculata* L. shows higher antibacterial and antifungal activity against bacterial and fungal pathogens such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger*. Phytochemical analysis showed that the antimicrobial activity of *C. auriculata* was due to the presence of Phytochemical compounds like alkaloids, carbohydrates, fixed oils & fats, tannins, gum & mucilage, flavonoids, saponins, saponins, terpenoids, lignin and sterols when compared with other three extracts *viz*., petroleum ether, ethyl acetate and methanol. The extract of *C. auriculata* showed maximum zone of inhibition at the concentration of 300µg/ml for antibacterial activity against bacterial pathogens while at the concentration of 300µg/ml showed maximum antifungal activity against fungal pathogens. The present study justifies the claimed uses of aerial parts of the *C. auriculata* in the traditional system of medicine to treat various infectious disease caused by the microbes.

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