ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

RESEARCH ARTICLE

In Vitro antimicrobial activity using ethanolic extract of flower and stem extract of *Cassia auriculata* linn.

Jayashree.V*, Anju K.C, M.P. Ragavendran, Dr. V. Ravichandiran
School of Pharmaceutical Sciences, Vels University, Pallavaram, Chennai
*Corresponding Author E-mail: **mailtovjayashree@gmail.com**

ABSTRACT:
The present study involves evaluating the invitro antimicrobial activity using ethanolic extract of flower and stem extract of *Cassia auriculata linn*. The ethanolic extract of both flower and stem extract of *Cassia auriculata* was found to possess antimicrobial activity against strains of two Gram negative organisms *Pseudomonas aeruginosa*, *Escherichia coli* and two Gram positive organisms *Staphylococcus aureus, Bacillus subtilis* and one fungal pathogen (*Candida albicans*). The antimicrobial activity of the flower and stem extracts of *Cassia auriculata* Linn. were evaluated by disc diffusion method and Minimum Inhibitory Concentration assay. The results obtained in the study indicates that the flower and stem extract using ethanol can be a potential source of antimicrobial agents.

KEYWORDS: *Cassia auriculata,* disc diffusion method, Anti-microbial activity, Minimum Inhibitory Concentration assay.

INTRODUCTION:
Herbal medicines was found to be known to man for centuries and they have used some of the traditional medicines to treat common infectious diseases[1]. Drug resistance to human pathogenic bacteria and fungus has been reported from all over the world[2]. Knowing the antimicrobial properties of plant extract can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted inorder to prove such efficiency[3,4]. The antimicrobial agents of plant origin are not associated with many side effects and hence it possess an enormous therapeutic potential to cure many infectious diseases[5]. *Cassia auriculata* belonging to the family Caesalpiniaceae was profoundly used in Ayurvedic medicine[6], known locally as "avaram" or Tanner's Cassia or Tanner's senna . It is a small shrub with smooth brown bark and it is a common plant in Asia, India and Srilanka. The leaves are useful as anthelmintic, good for ulcers, leprosy and skin diseases. The flowers have been used in urinary discharges, diabetes and also for throat infection. The fruit is useful in thirst and in vomiting. The seed is useful in diabetes, dysentery and chronic conjunctivitis. The bark is considered as astringent[7,8,9].

Received on 21.07.2015 Modified on 30.07.2015
Accepted on 07.08.2015
Research J. Pharm. and Tech. 8(7): July, 2015; Page 901-905
DOI: 10.5958/0974-360X.2015.00146.8

In the present investigation, an attempt has been made to enrich the knowledge of antimicrobial activity of ethanolic extract of the flower and stem parts of *C. auriculata* Linn.

MATERIALS AND METHODS:
Preparation, collection and extraction of plant material
The fresh plant material, stem and flower of *Cassia auriculata* was collected from Thiruvanamalai district and Potheri, Chennai district, Tamilnadu, India. The plant was identified based upon the organoleptic and macroscopic examination of fresh flower and stem and authenticated by Prof P. Jayaraman director of Institute of Herbal Botany ,Plant Anatomy Research Center, bearing the reference number PARC/2015/3061. The collected stems and flowers of *Cassia auriculata* is dried thoroughly under shade and powdered mechanically into a coarse powder. The powdered stem and flower were kept in airtight container until time of use.

The flower extract was carried out by continuous hot percolation method using Soxhlet apparatus[10]. The solvent used was 95% ethanol. About 50g of powder was extracted with 400ml of solvent for 72 hours. The extract was concentrated to dryness under controlled temperature between 40-50ºC. The percentage yield of

the *Cassia auriculata* flower extract (CAFE) was 16.59% w/v.

The stem extract was carried out by continuous hot percolation method using Soxhlet apparatus[10]. The solvent used was 95% ethanol. About 50g 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* stem extract (CASE) was 12.48% w/v .

Phytochemical Screening of the extract

The ethanolic extract of stem and flower were subjected to qualitative test for the identification of various chemical constituent (alkaloids, flavonoids, glycosides, proteins, saponins, terpenoids, tannins and phenols)[11,12,13].

Antimicrobial Activity

Test Organisms[14]

Two Gram negative *Pseudomonas aeruginosa, Escherichia coli* and two Gram positive *Staphylococcus aureus, Bacillus subtilis* bacterial pathogens and one fungal pathogen (*Candida albicans*) were used for *in vitro* antimicrobial activity. These selected pathogenic strains were obtained from Microbiological Division (Jayagen Biologics Analytical Laboratory, Chennai).

Preparation of Inoculum

Stock cultures were maintained at 4°C on Nutrient agar Slant. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing nutrient broth , that were incubated at 24hrs at 37ºC. The Assay was performed by agar disc diffusion method.

For Antibacterial Activity

Take 100ml of distilled water in a conical flask and to this add 3.8gm of Mueller Hinton agar (Hi media) and 1 gm of agar for fast solidification. The flask was tightly plugged with cotton and wrapped. It was then autoclaved at 15lbs pressure for 20mins. After sterilization the media was bought to laminar chamber and add antifungal agent Clotrimazole to avoid bacterial contamination, when the media is half cooled. Then the media was poured into the petridish for solidification.

Invitro Antibacterial Activity

The antibacterial activity of the flower and stem extracts of *Cassia auriculata* Linn. was determined by disc diffusion methods (CLSI 2000). About 25 mL of molten Mueller Hinton agar was poured into a sterile Petri plate (Himedia, Mumbai, India). The plates were allowed to solidify, after which 18 h grown (OD adjusted to 0.6) 100 µl of above said pathogenic bacteria cultures were transferred onto plate and made culture lawn by using sterile swab. After five min setting of the pathogenic bacteria, drug impregnated 5 mm discs were placed on to the media. The test samples were dissolved in DMSO (5%) and loaded on to discs with various concentrations such as 50 µg/well, 100 µg/well, 150 µg/well and 200 µg/well. The 5% DMSO loaded disc served as control. The plates were incubated at 37°C in a 40 W florescent light source (~ 400 nm) for 24 h. The antibacterial activity was determined by measuring the diameter of the zone of inhibition around the well using antibiotic zone scale (Himedia, Mumbai, India) [15]

For Antifungal Activity

Preparation of Fungal Culture Media

Take 100ml of distilled water in a conical flask and to this add 6.5gm of Sabouraud Dextroseagar (Hi media) and 1 gm of agar for fast solidification. The flask was tightly plugged with cotton and wrapped. It was then autoclaved at 15lbs pressure for 20mins. After sterilization the media was bought to laminar chamber and add antibacterial agent ampicillin to avoid bacterial contamination , when the media is half cooled. Then the media was poured into the petri dish for solidification.

Invitro Antifungal Activity

The antifungal activity was determined by disc diffusion methods (CLSI 2000). About 25 mL of molten Sabouraud Dextroseagar was poured into a sterile Petri plate (Himedia, Mumbai, India). The plates were allowed to solidify, after which 18 h grown (OD adjusted to 0.6) 100 µl of above said pathogenic fungi cultures were transferred onto plate and made culture lawn by using sterile swab. After five min setting of the organism, drug impregnated 5 mm discs were placed on to the media. The test samples were dissolved in DMSO (5%) and loaded on to dics with various concentrations such as 50 µg/well, 100 µg/well, 150 µg/well and 200 µg/well. The 5% DMSO loaded disc served as control. The plates were incubated at37°C in a 40 W florescent light source (~ 400 nm) for 24 h. The antifungal activity was determined by measuring the diameter of the zone of inhibition around the well using antibiotic zone scale (Himedia, Mumbai, India) [15]

MIC Determination using Microbroth Dilution Assay

Based on the antimicrobial activity of the stem and flower extract that showed active against *Staphylococcus aureus* very remarkably, Since both extract were taken individually for MIC determination using the standard procedure of CLSI (2012). The microbroth dilution assay was performed to check the MIC for both extract individually. The *S.aureus* has been grown in cation adjusted Mueller Hinton broth and adjusted 5×10^4cfu/well. The doubling concentration of the test sample was introduced as 6.25, 12.5, 25, 50, 100, 200, 400 and 800µg/well. Incubate the inoculated macrodilution tubes or microdilution trays at 35±2°C for 16 to 20 hours in an ambient air incubator within 15

minutes of adding the inoculum. To prevent drying, seal each tray in a plastic bag, with plastic tape, or with a tight-fitting plastic cover before incubating. The MIC is the lowest concentration of antimicrobial agent that completely inhibits growth of the organism in the microdilution wells as detected by the unaided eye[16].

RESULTS:

The ethanolic extract of stem and flower revealed the presence various chemical constituents like alkaloids, flavonoids, glycosides, proteins, saponins, terpenoids, tannins and phenols.

Antimicrobial activity

The results of antimicrobial activity of ethanolic extract of flower and stem by well diffusion method was shown in Table 1 and 2. The antimicrobial activity was measured by zone of inhibition (mm).

The antibacterial activity of ethanolic extract of flower showed better growth of inhibition against *Staphylococcus aureus* (16 mm at 200µg/well) whereas ethanolic extract of stem showed better growth of inhibition in Escherichia coli (16mm at 200µg/well) when compared to the other test organisms.

The antifungal activity of ethanolic extract of flower showed more growth of inhibition (28mm at 200µg/well) when compared to ethanolic extract of stem (15mm at 200µg/well).

Table 1: Antimicrobial activity of Flower extract

Name of the organisms	Zone of the Inhibition (mm)			
	50µg/ well	100µg/ well	150µg/ well	200µg /well
Staphylococcus aureus	9	11	14	16
Bacillus subtilis	8	10	12	14
Pseudomonas aeruginosa	-	8	11	14
Escherichia coli	7	9	12	15
Candida albicans	13	17	21	28

Table 2: Antimicrobial activity of Stem extract

Name of the organisms	Zone of the Inhibition (mm)			
	50µg/ well	100µg /well	150µg/ well	200µg/ well
Staphylococcus aureus	7	9	11	14
Bacillus subtilis	-	-	8	12
Pseudomonas aeruginosa	7	8	9	11
Escherichia coli	7	9	12	16
Candida albicans	7	9	12	15

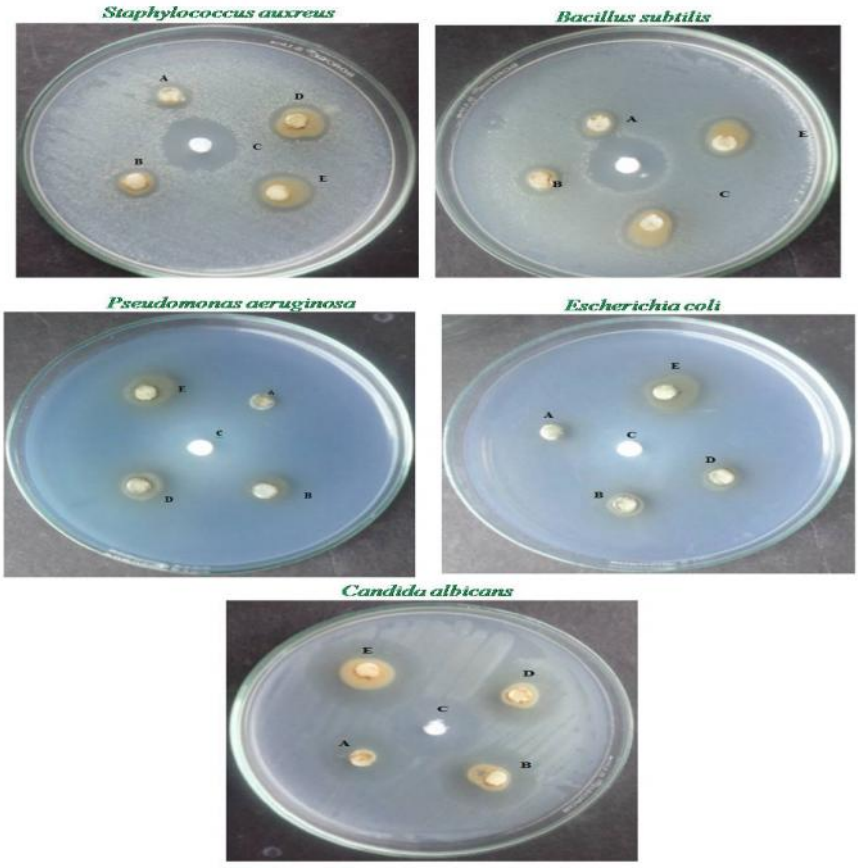

Figure 1: Inhibition of microbial growth using ethanolic flower extract of *C.auriculata* by Disc diffusion method. C: Control; A: 50 µg/disc; B: 100 µg/disc; D: 150 µg/disc; E: 200 µg/d

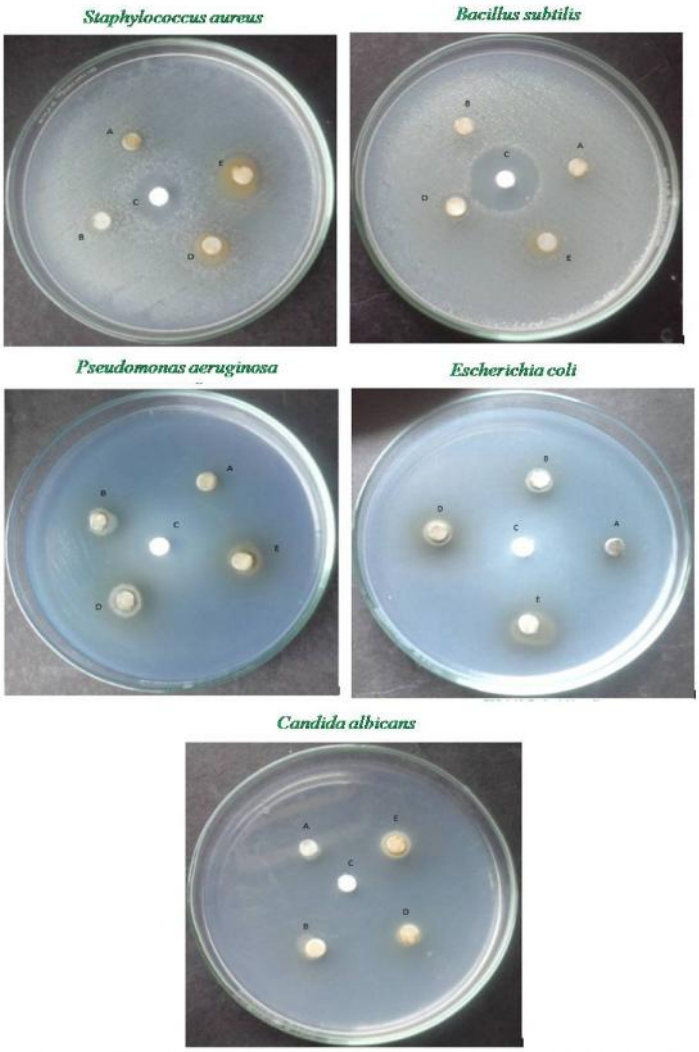

Figure 2: Inhibition of microbial growth using ethanolic stem extract of *C.auriculata* by Disc diffusion method. C: Control; A: 50 µg/disc; B: 100 µg/disc; D: 150 µg/disc; E: 200 µg/d

MIC assay

The MIC determination of the both stem and flower showed minimum inhibitory concentration level of increased concentration. Ethanolic extract of flower showed MIC at **200 µg** killed entire *S. aureus* inoculated well after 20 h of incubation whereas ethanolic extract of stem showed MIC at **400 µg** that killed the entire 5×10^4 CFU/well at 20 h of incubation.

DISCUSSION:

The medicinal property and pharmacological action of C*assia auriculata* is well used in the Indian traditional medicine. These plants are known to contain various active principle of therapeutic value and to possess biological activity against a number of diseases.

Cassia auriculata (linn) belonging to the family Caesalpiniacea, is commonly known as “Avaram” in Tamil, “Tangedu” in Telugu, “tanner’s cassia” in English and “avara” in Malayalam. The flower and stem parts was taken for the present study and ethanolic extract were prepared. The work was planned to investigate the antimicrobial activity of flower and stem extract using various organisms such as two Gram negative organisms (*Pseudomonas aeruginosa and Escherichia coli*) and two Gram positive organisms, (*Staphylococcus aureus* and *Bacillus subtilis*) and one fungal pathogen (*Candida albicans*).

The ethanolic extract of flower and stem were prepared and their yield were noted. The preliminary phytochemical test were performed on the ethanolic extract of stem and flower and showed the presence of various chemical constituents like alkaloids, flavonoids, glycosides, proteins, saponins, terpenoids, tannins and phenols.

The zone of inhibition were measured after 24 hours of incubation and the result were tabulated (Table-1and2). The ethanolic flower extract showed more activity in *Staphylococcus aureus* (zone of inhibition-16 mm at 200µg/well and ethanolic extract of stem showed more activity in *Escherichia coli* (zone of inhibition-16mm at 200µg/well) when compared to other test organisms. The antifungal activity of ethanolic extract of flower showed more inhibition of growth (28mm at 200µg/well) when compared to ethanolic extract of stem (15mm at 200µg/well).

REFERENCES:

1. Devados Kumarasamy Raja, Nattanmai Sundararaman Jeganathan, Rajappan Manavalan. In vitro antimicrobial activity and phytochemical analysis of Cassia auriculata Linn. **International Current Pharmaceutical Journal.** 2(6); 2013: 105-108.
2. Piddock, K.V.J. and R. Wise. Mechanisms of resistance to quinolones and clinical perspective. **J. Antimicrobial Chemotherapy.** 23; 1989: 475-483.
3. Shapoval EES, Silveira SM, MirandaML, Alice CB and Henriques AT. Evaluation of some pharmacological activities of Eugenia uniflora**. J Ethnopharmacol.** 44; 1994:136-142.
4. Jansen AM, Cheffer JJC and Svendsen AB. Antimicrobial activity of essencial oils: a 1976-1986 literature review. **Aspects of test methods. Planta Med**.40; 1987:395-398.
5. Iwu, M.W. and A.R. Ducan and C.O. Okungi. New antimicrobials of plant orgin. In: Janik J. (ed.) Perspectives on new crops and new uses. 1999; *ASHS press, Alexandria*. VA: 457-462.
6. Kainsa, S., Kumar, P. and Poonamrani. Pharmacological potentials of *Cassia auriculata* and *Cassia fistula* plants: A **Review, Pakistan Journal of Biological Sciences**. 9(15); 2012: 408-417.
7. Senthilkumar, P.K., Reetha, D. Isolation and identification of antibacterial compound from the leaves of Cassia auriculata. **European Review for Medical and Pharmacological Sciences.** 15(9); 2011: 1034-1038.
8. Parmar Y, Chakraborthy GS. Evaluation of Cassia auriculata leaves for its potent biological activity carried out. **J Pharmacol online.** 2; 2011:128–33.
9. Indira S and Lakshmi M. Antibacterial activity of *Cassia auriculata* linn against some pathogens isolated from wound. **International Journal of Chemical and Pharmaceutical Sciences.** 2013, 4 (2)
10. V. Subhadradevi, K. Asokkumar, M. Umamaheswari, A. T. Sivashanmugam, J.R.Ushanandhini and P. Jagannath. Antimicrobial Activity of Leaves and Flowers of *Cassia auriculata* linn. **Bangladesh J. Sci. Ind. Res.** 46(4);2011: 513-518.
11. Dr. C.K. Kokate. Practical Pharmacognosy, 4th edition, 1994,Pg.108-111.
12. Mohamed Sham Shihabudeen, H., D. Hansi Priscilla and Kavitha S.S. Thirumurugan. Antimicrobial activity and Phytochemical analysis of selected Indian folk medical plants. **International Journal of Pharma Science and Research (IJPSR).** 1(10);(2010): 430-434.
13. Harborne, J. B. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 2nd Edition, J. B. Harborne Publishers, London, Chappman and Hal, 1998,1-32.
14. C. Anushia, P. Sampathkumar and L. Ramkumar. Antibacterial and Antioxidant Activities in *Cassia auriculata*. **Global Journal of Pharmacology**. 3(3); 2009:127-130.
15. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests 7th edition. Approved Standard. NCCLS document M2-A7. National Committee for Clinical Laboratory Standards, Villanova, PA. 2000.
16. Clinical Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard -Ninth Edition, CLSI, Wayne, Pennesylvania, 2012.