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ANTIBACTERIAL ACTIVITY OF *CASSIA AURICULATA* AND *ALOE VERA* AGAINST PATHOGENIC BACTERIA ISOLATED FROM SPUTUM AND PUS SAMPLES

Balaji M* Rajeswari.K* Rajkumar . S**
**Department of Biotechnology, Rathnavel Subramanium College of Arts and Science, Coimbatore.*
***Microtech diagnostic center, Coimbatore.*

Introduction

Infectious diseases are the world's major threat to human health and account for almost 50,000 deaths every day. The situation has further been complicated with the rapid development of multidrug resistance by the microorganisms to the antimicrobial agents available. Diseases are the major causes of death in the developing countries and accounts to 50% of it. The extensive use of the antibiotics to control these diseases has led to the emergence of multidrug resistance (Westh et al., 2004). For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant extract for medicinal treatment has become popular when people realized that the effective life span of antibiotic is limited and over prescription and misuse of traditional antibiotics are causing microbial resistance (Alam et al., 2009). At present, nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly from plants and their extracts dominate in homeopathic or ayurvedic medicines (Murugesan et al., 2011).

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 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. Every part of the plant is valuable in medicine for ulcers, leprosy and liver disease. The plant can also be used as an antidiabetic, hypolipi-demic 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 (Tomoko *et al.*, 2000).

Aloe, is a genus containing about four hundred species of flowering succulent plants belonging to *Lileaceae* family . Aloe vera is a typical xerophyte with thick fleshy, strangely cuticularized spiny leaves. The gel is a watery-thin, viscous, colorless liquid that contains anthraquinone glycosides, glycoprotein, gamma-lanoline acid, prostaglandins and mucopolysaccharides that are mainly responsible for the antibacterial, antifungal as well as its antiviral activity (Shafi et al., 2000).The search for newer sources of antibiotic is a global challenge preoccupying research institutions, pharmaceutical companies and academic institutions, since many infectious agents are becoming resistant to synthetic drugs (Latha and Kannabiran, 2006).The present work was aimed to study the prevalence of the drug resistance among Gram positive and Gram negative bacteria isolated from clinical samples (Pus and Sputum) obtained from various clinical lab at Tirupur city and surrounding areas and investigates the antibacterial activity of different parts of *Cassia* and *Aloe vera* in different concentration, in comparison with standard drugs.

Materials and Methods

Sample Collection

Samples for the study were collected from the patients of different Hospital and Microbiology laboratories at Tirupur, Tamilnadu, India. Clinical specimens such as sputum (100 samples) and, pus swabs (100 Samples) were collected aseptically by using sterile cotton Swabs.

Wound (Pus) swabs

Swabs from pus, abscesses, as well as lesions were taken on two sterile cotton swabs (culture) from the same site of infection. It was cultivated immediately without delay. Swabs were cultured on Blood agar, MacConkey agar. The plates were incubated under 37°C for 18-24 hours.

Sputum Samples

Sputum culture is a test to detect and identify bacteria or fungi that are infecting the lungs or breathing passage. Sputum is coughed up from the bronchi and therefore, mixed with organisms in the throat and mouth. Sputum sample was expectorated directly into a sterile container. The patients were instructed for washing the mouth before collection of sputum sample and bring it from deep cough (tracheobronchial sputum). The sputum samples were cultured immediately within few hours of being collected on Blood agar medium, MacConkey agar. Sterile saline (8.5%) were added to sputum specimen and agitate to

free it from adherent saliva. Then, the saline was removed with sterile pasteur pipette. One to two drops of the specimen were placed onto the culture plates and on the glass slide, and then streaked for isolation.

Identification of Bacterial Pathogens

Bacterial pathogens were identified by conventional biochemical methods according to standard microbiological techniques. The following biochemical tests were performed for the identification of bacteria Gram staining, Indole, MRVP test, TSI, Citrate, Urease, Sim agar and Mannitol Motility Media (MMM).

Kirby-Bauer Disk Susceptibility Test

One of the most useful and widely used laboratory tests for antimicrobial susceptibility. The pure cultures of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas* sp which was obtained from patients sample was inoculated into a sterile Peptone broth. The tubes were incubated at 37±1ºC for 2-4 hours. A sterile cotton swab was dipped into the broth culture tube. The swab was streaked on the surface of the nutrient agar medium in 3 planes; horizontal, vertical and diagonal (according to Kirby-Bauer method) (Bauer et al., 1966). After the inoculum had dried (3-5 min), the discs (Vancomycin, Cefotaxime, Ofloxacin, Ceftriaxone, Ceftazidime, Tetracyclines, Amikacin, Chloramphenicol, Gentamicin, Ampicillin, Erythromycin, Rifampicin, Neomycin, Co-trimoxazole, Pencillin G, Cefazolin, Ceflexin and Nalidixic acid) were placed individually on the agar surface with a sterile forceps and are then gently pressed down onto the agar surface to provide uniform contact. The plates were incubated aerobically for 18-24hours at 37±1ºC.The diameter of the inhibition zone around each disk was measured by a ruler from the back of the plates.

Preparation of Plant Extract

Fresh plant materials Cassia (both leaf and Flower) and Aloe vera were washed; shade dried and then powdered using the blender and stored in air tight bottles. 50gm of powdered material was extracted with 300 ml of methanol solvents (12 hour each). All the extracts were evaporated under vacuum. Different concentrations were prepared by dissolving the extract in DMSO.

Antibacterial Activity of Plant Extract

Discs of 6mm diameter were impregnated with (100, 500, 1000 mg) of the plant extract previously dissolved in DMSO. The discs were evaporated at 37°C for 24h. The stains *E.coli* and *Staphylococcus aureus* were utilized for the antibacterial susceptibility test.

Result

Isolation of Clinical Pathogens

In the present study totally 200 different clinical samples were collected and processed for isolation of pathogenic bacteria. The two types of clinical samples included pus and sputum. Out of total samples analyzed 63 samples showed the presence of infection while in 137 samples no growth of organisms was seen on culture medium (Table 1). The latter may be because of i) at the time of sample collection the patient may be already on a suitable antibiotic treatment or ii) the patient may be suffering from infection by anaerobes / viral agents. A list of clinical samples collected and number of organisms isolated from respective samples is given in Table 1. In the present study, out of the total clinical isolates (n = 200), Gram negative organisms were dominant compared to Gram positive organisms in the sputum and pus sample.

Table 1 Profile of different sample-wise classification of infectious organisms isolated from various clinical samples

Name of the Organism	Pus sample	Sputum sample
Escherichia coli	2	3
Staphylococcus aureus	13	-
Pseudomonas sp	1	6
Enterococcus	3	2
Klebsiella pneumoniae	2	6
Streptococcus pneumoniae	5	1
Acinetobacter sp	2	4
Proteus sp	2	2
Enterobacter sp	1	3
Candida sp	1	4
Total	32	31

Identification of Bacteria

The strains were identified based (Table 2)on biochemical characteristics. Strains *Staphyloccus* and *Streptococcus* were identified based on Gram's reaction, Catalase test and colony morphology on Mannital salt agar medium. Strain *Staphyloccus* gave positive result for catalase and produce golden yellow colonies on mannitol salt agar medium, whereas *streptococcus* gave negative for both the test. Both *Staphylococcus* and *Streptococcus* produce beta hemolysis on blood agar medium.

Table 2 Biochemical Test for the Identification of Bacteria

Name of the Bacteria	Name of the test								
	Gram staining	Indole test	MR	VP	Citrate	TSI	Urease	MMM	Sim agar
E.coli	-	+	+	-	-	A/A	-	+	+
Klebsiella sp	-	-	-	+	+	A/A	-/+	+/-	-
Entero bacter sp	-	+	-	+		A/A	-	+	-
Pseudomonas sp	-	-	-	-	+	K/K	-	-	+
Acinetobacter sp	-	-	-	-	+	K/K	V	-	-
Proteus sp	-	-	+	-	-	K/A	+	+	+

MR-Methyl Red; VP-Voges Proskauer; TSI –Triple Sugar Iron ; MMM-Mannitol motility media; A/A- Acid slant and Acid butt; K/K-Alkaline slant/Alkaline butt; K/A-Alkaline Slant/Acid butt

Antimicrobial activity against *Staphylococcus aureus*

As shown in Table 2, Gentamicin, Chloramphenicol and Tetracyclines had the highest inhibition zone (21 mm) followed by Ofloxacin, Amikacin and Neomycin (20 mm), while there was no effect of Ceftazidime, Ampicillin, Penicillin G and Cefazolin against *S.aureus*.

Antimicrobial activity against *Escherichia coli*

Using disc plate method the effectiveness of a range of antibiotics was determined against E. coli (Table 3 and Figure 1). chloramphenicol was showed the highest inhibition zone against E. coli (24 mm), while it was sensitive to tetracyclines, ofloxacin, ampicillin, cefazolin, nalidixic acid and co-trimoxazole.

Antimicrobial activity against *Pseudomonas aeruginosa*

Amikacin, Ceftazidime and Gentamicin were showed the strongest activity against P. aeruginosa while the rest antibiotics had no effect as shown in Table 3.

Table 3 Evaluation of antibiotics activity against *S. aureus, E. coli* and *P. aeruginosa*

Antibiotics	***Staphylococcus aureus***	***E.coli***	***P. aeruginosa***
Vancomycin	15mm	*	*
Cefotaxime	11mm	8mm	0mm
Ofloxacin	20mm	0mm	0mm
Ceftriaxone	12mm	9mm	0mm
Ceftazidime	0mm	11mm	9mm
Tetracyclines	21mm	19mm	*
Amikacin	20mm	10mm	17mm
Chloramphenicol	21mm	24mm	*
Gentamicin	21mm	7mm	8mm
Ampicillin	0mm	0mm	*
Erythromycin	17mm	*	*
Rifampicin	19mm	*	*
Neomycin	20mm	14mm	0mm
Co-trimoxazole	10mm	0mm	*

Pencillin G	0mm	*	*
Cefazolin	0mm	0mm	*
Ceflexin	10mm	7mm	0mm
Nalidixic acid	*	0mm	*

*= Have not been tested. mm= millimeter

Fig 1 Inhibition zone (mm) of some antibiotics against *E.coli, S.aureus, pseudomonas*.

Evaluation of Plant Extracts Bioactivity

The result in Table 4 revealed that, the disc diffusion method evaluated the antimicrobial activity of plant extracts better than the well diffusion method (data not shown) against *E. coli*. Cassia auriculata (leaves) and Cassia auriculata (flower) (extracted by methanol) were showed the highest effect against *S.aureus* with a zone of inhibition = 15mm(1000mg), 13mm(500mg), 10mm(300mg). No antimicrobial activity was observed by Cassia auriculata (leaf). The aloe vera extract showed minimum effect agaist *S.aureus* with a zone of inhibition = 11mm(500mg), 9mm(300mg), 5mm(100mg). Aloe vera (gel) (extracted by methanol) were showed the highest effect against *E. coli* with a zone of inhibition 20mm(1000mg), 10mm(500mg), 8mm(300mg). No antimicrobial activity (Table 4) was observed by Cassia auriculata (leaves) and Cassia auriculata (flower).

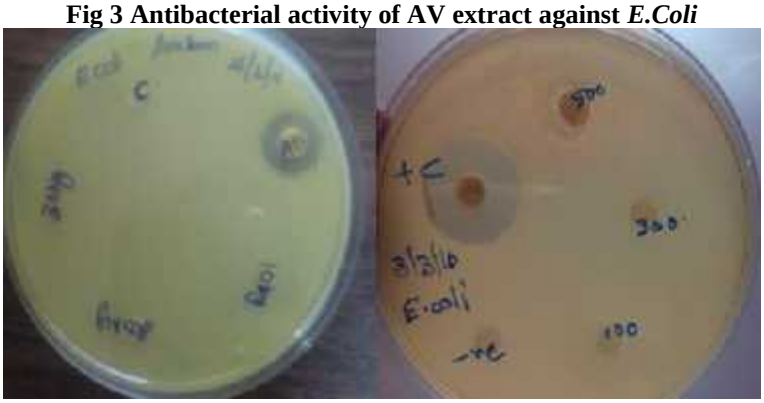
Table 4 Antimicrobial Activity of Plant extracts on *S.aureus* and *Escherichia coli* by disc diffusion method

Bacteria	Concentration (mg/3ml)	Zone of inhibition (mm)	Plant Extract
Staphylococcus aureus	300	15	AF
	500	13	AF
	1000	10	AF
	100	5	AV
	300	9	AV
	500	11	AV
Positive control	50	30	
Negative control	-	-	
Escherichia coli	300	8	AL
	500	10	AL
	1000	20	AL
Positive control	50	26	
Negative control	-	-	

Fig 2 Antibacterial activity of AF ,AL and AV extract against *S.aureus*

Positive control –Tetracyclin; Negative control - DMSO

Fig 3 Antibacterial activity of AV extract against *E.Coli*



Positive control –Tetracyclin; Negative control - DMSO

Discussion

Plants are an important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the in vitro antimicrobial activity assay and in recent years several reports are available on antimicrobial activity of plant extracts on human pathogenic microorganisms (Brantner, A. and E. Grein, 1994).Anushia *et al.* 2009 checked the antibacterial and antioxidant activities in *Cassia auriculata* and found that organisms i.e., *S. aureus* and *E. coli* both at the concentration of 64mg/ml. In the present study, methanol extracts of *Cassia auriculata showed* significant zone of inhibition against *Escherichia coli*, and *Staphylococcus aureus* at 100 mg/3 ml concentration.

Maneemegalai and Naveen (2010) studied the antibacterial activity of the microorganisms; *Staphylococcus aureus, Enterococcus fecalis, Bacillus subtilis, Salmonella typhi, Salmonella paratyphi A, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Vibrio cholera* and *Shigella dysenteriae*. The maximum activity was observed against all organisms except *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Presence of phytochemicals such as terpenoids, tannins, flavonoids, saponin, cardiac glycosides and steroids were observed and they declared that *Cassia auriculata* is observed to have antibacterial activity and can be used for medicinal purposes. Perumalsamy and nacimuthu 2000, reported that the leaf extracts of *Cassia auriculata* exhibited significant broad spectrum activity against *Bacillus subtilis* and *Staphylococcus aureus*.

Antimicrobial activity of *Cassia auriculata* flower extract has been observed by (Narayanan *et al.* 2007).The extract of *Cassia auriculata* was found to have potent microbicidal activity against the *E. coli* in poultry Prakash, S.K., 2006. Mangena 1999, reported that the Aloe vera gel was found to be very effective against *Staphylococcus aureus*. As the Aloe vera gel is rich in a wide variety of secondary metabolites, such as anthraquinone glycosides, glycoproteins, gamma-lanoline acid, prostaglandins and mucopolysaccharides, these are mainly responsible for the antimicrobial activity (Shafi N et al., 2000).

Agarry *et al*., (2005) compared the antimicrobial activities of the gel and leaf of *Aloe vera* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Trichophyton mentagraphytes*, *T. schoeleinii*, *Microsporium canis* and *Candida albicans*. The result showed that both the gel and the leaf inhibited the growth of *S. aureus* (18.0 & 4.0 mm). Only the gel inhibited the growth of *I. mentagrophytes* (20.0 mm), while the leaf possesses inhibitory effects on both *P. aeruginosa* and *C. albicans*. Similarly, the antimicrobial activity of the *Aloe vera* juice against Gram-positive bacteria (*Mycobacterium smegmatis*, *Enterococcus faecalis*, *Micrococcus luteus* and *Bacillus sphericus*), Gram-negative bacteria (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *E. coli* and *Salmonella typhimurium*) and *Candida albicans* were also studied.

Conclusion

From our results it can be concluded that Cassia and Aloe vera gel extract possesses compounds with antimicrobial properties which can be used as antimicrobial agents in new drugs for the therapy of infectious diseases in humans. The results of the present study thus explains the use of this plant in folk medicine for the treatment of various diseases whose symptoms might involve microbial infections and underline the importance of ethno botanical approach for the selection of Cassia and Aloe vera in the discovery of new bioactive compounds. These plants could be a source of new antibiotic compounds being nontoxic and less expensive than the allopathic drugs.

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