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Antibacterial activity of leaf extract of *Cassia alata* separated by soxhlet extraction method

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ABSTRACT

Cassia alata belongs to the family Caesalpiniaceae. Leaf extract of this plant have been reported that it posses medicinal property and used against ringworm, scabies, ulcers and other skin disease such as pruritis, eczema and itching. Many reports are available on the antimicrobial activity of the plant extracts. The aim of the present work was to estimate the antibacterial activity of the of leaf extract of *Cassia alata* produced by soxhlet extraction method. The solvent selected for this study was hydro-alcohol. The plant extract was tested against pathogenic gram positive and negative bacterial strains viz., *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* and *Serratia marcescens* by agar disc diffusion method. The concentration was made as 5 μ l, 10 μ l and 20 μ l to check the bacterial growth and the activity was found good with the high concentration. It was also compared with standard antibiotics (Rifamycin, Amoxyclav). This study showed that the plant extract comparatively work better than the standard drug, Amoxyclav in order to control the pathogenic bacteria.

Keywords: *Cassia alata*, Antibacterial activity, Disc diffusion method, Soxhlet extractor.

INTRODUCTION

Utility of medicinal plants as traditional medicine is one of the common practices in India due to their wide pharmacological activities. Traditional medicines are being used at the primary health care level by many developed and developing countries. More than 80% of world's population depends on plants for primary health care needs [1]. A study by WHO on conventional antimicrobial drugs such as benzyl penicilin, gentamycin, metronidazole, ampicilin, phenoxy methyl pencilin and ciprofloxacin revealed that these drugs are no longer very effective against bacterial pathogens [2]. Some drugs may also have side effects and cause other dysfunctions in the body. This situation urgently forced scientists for searching drugs which are inexpensive, safe, biodegradable and less side effects [3]. Traditional medicine plays a significant role in the health care system of developing countries, especially in Asia including India, Bangladesh, China, Burma and Sri Lanka. In other way, medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value [4]. *Cassia alata* Linn was native to Ghana and Brazil, but it is now widely distributed in the Americas and all over Africa, Nigeria inclusive India. Different parts and constituents of the plant were reported to exhibit several therapeutic properties, such as antibacterial, antifungal, antimicrobial and analgesic. The leaves of this plant are used in the treatment of ringworm. The plant is traditionally acclaimed to be effective in treating skin infections in man and animals [5]. *C. alata* leaf is also credited for the treatment of haemorrhoids, constipation, inguinal hernia, intestinal parasitosis,

blennorrhagia, syphilis and diabetes [6]. In Peruvian herbal medicine systems, *Cassia alata* is called *retama* and the flowers are prepared in an infusion to treat urinary infections and used to increase urination; the leaves and stems are prepared in a decoction for ascaries and herpes ulcers. The leaves extracts or capsules are used to treat for liver problems, anemia, dyspepsia, menstrual problems and high fevers. As well as supporting scientific thinking, an investigation with ancient approaches to the current healthcare issue of anti-bacterials. In our present study, the separation of leaf extract was made from the medicinal plant, *Cassia alata* by soxhlet extraction method and the extract was employed in vitro as antibacterial agent in order to treat with few pathogenic bacteria with a comparison with Rifamycin and Amoxyclav drugs.

MATERIALS AND METHODS

Collection of Plant material

The healthy and fresh leaf of the plant *Cassia alata* was collected from Villianur of Puducherry region. The collected leaves were washed in tap water for 2-3 times. The excess water was removed by sterilized blotting paper.

Preparation of Plant extract

The plant material was dried in the shade for 10 to 15 days. The dried plant material was crushed by grinder without adding any solvent [7] into it. The powder of the plant materials were initially defatted with petroleum ether followed by 100 ml of hydro-alcohol by using a soxhlet extractor for 24 hours and dried in desiccators. Once the process was finished, the hydro-alcohol was evaporated using a rotary evaporator leaving a small yield of extracted plant material (about 2 to 3 ml) in the glass bottom flask. The hydro-alcoholic extract yielded a dark greenish residual mass. The extracts were then kept in sterile bottles and in refrigerator condition. The extract was preserved at 2-4°C [1] until further experiment.

Antibacterial activity

The leaf extract were dissolved in DMSO (dimethyl sulfoxide) [8, 9] for few minutes with same concentration together. Three sets of dilution (5µl, 10µl, 20µl) of *Cassia alata* extract were prepared in milli-Q water. Mueller Hinton Agar (MHA) was used for bacteria bioassay. MHA was prepared by dissolving 38g in 1000ml of distilled water and brought to boil to completely dissolve. Sterilization was achieved by autoclaving at 121°C for 15 minutes [8]. Extract were screened for their antibacterial activity against the gram-positive (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*) and gram negative (*Escherichia coli*, *Serratia marcescens* and *Proteus vulgaris*). MHA plates were prepared and bacterial strains were inoculated by cotton swab. The discs were prepared at concentration (5µl, 10µl, 20µl) and placed over the plates with the antibiotics discs of Rifamycin and Amoxyclav. The plates were incubated at 37°C for 24 hours and the zone of inhibition were measured [1] and recorded later on.

RESULTS AND DISCUSSION

The results of the present study observed that the crude extract prepared from the leaves of *C. alata* had inhibitory activity against all the bacterial strains (Table 1). *Pseudomonas aeruginosa* was resistant to the extracts and also to the antibiotics. The antibacterial activity of the leaf extract was compared with standard drugs. *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* were found sensitive to the extracts in comparison to *Proteus vulgaris* and *Serratia marcescens* (Fig 1 & 2). The result showed that the extract of *Cassia alata* where found to be more effective against the bacterial species. Amoxyclav was found impotent against *Bacillus* and *Serratia* but the extract was found effective against all the strains other than *Pseudomonas*. 10µl dilution of the leaf extract was found suitable to control the growth of the bacteria. The ethanol extract of leaf and flower of *Spathodea campanulata* was investigated by Kowti et al [10] for antimicrobial activity at 10 mg/ml concentrations by using Kirby-Bauer disc diffusion method against gram positive and gram negative organisms like *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas sps*, *Salmonella typhimurium*, *Bacillus subtilis*, *Staphylococcus aureus*, *Vibrio cholera* [10]. After incubation for 24 hrs, the zone of inhibition was compared with standard antibiotics gentamycin and streptomycin (10 µg/ disc). From the dose dependent study it was observed that the ethanol flower extract was more potent than leaf extract [10,11]. Flavonoids and tannins present in the both ethanol extract may be responsible for the antimicrobial activity [10]. Extracts of mature leaf of marine plant, *Avicennia marina* and tender leaf of *A. officinalis* exhibited considerable antibacterial activity against tested bacterial strains [2]. Pushpa et al [2] also opined that in addition to above tested mangrove plant species, other mangrove plants can be used to identify and isolate antibacterial components against pathogenic bacteria species. Evidence proved that, *Exoecaria agallocha*,

Lumnitzera racemosa and *Rhizophora apiculata* had antibacterial activity against *Staphylococcus* sp. and *Proteus* sp. Mhatre et al [8] elaborated the antibacterial activity of ointments was tested and ethanol and methanol extract of unripe fruit using soxhlet apparatus showed good antibacterial potential. They also concluded that the unripe fruit showed maximum antibacterial activity against *S. aureus* compared to leaves, ripe fruit and stem. Soxhlet extract hydro-alcohol extract showed maximum zone of inhibition compared to methanol and aqueous extract, which was proved by previous authors [2,8,11]. The antibacterial activity was retained after the formulation of extract.

Table1: Antibacterial efficacy of *Cassia alata* leaf extract against six bacterial pathogens.

Bacterial pathogens	Dilutions of Soxhlet leaf extracts of <i>Cassia alata</i>			Antibiotics Rif: Rifamycin, Amc: Amoxycylav	
	5 μ l	10 μ l	20 μ l	Rif	Amc
<i>Bacillus subtilis</i>	10	13	8	17	0
<i>Pseudomonas aeruginosa</i>	0	0	0	9	0
<i>Staphylococcus aureus</i>	16	12	8	25	17
<i>Proteus vulgaris</i>	10	13	8	15	8
<i>Escherichia coli</i>	11	16	10	23	20
<i>Serratia marcescens</i>	8	7	8	13	0

Fig 1: Susceptibility/resistnace of bacterial strains towards leaf extract and antibiotics studied together

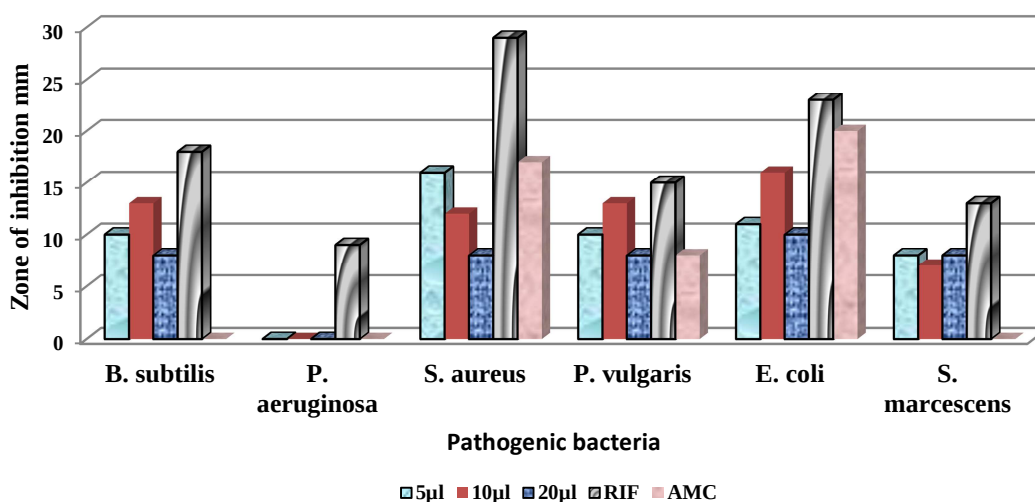
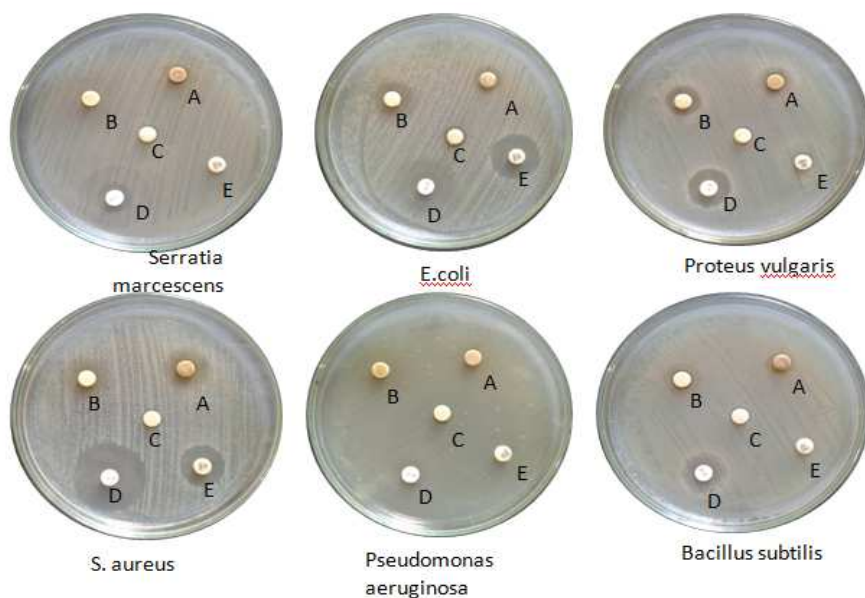


Fig 2: Zone of inhibition made by leaf extracts and antibiotics against bacterial pathogens
A: 5 μ l, B: 10 μ l, C: 20 μ l of leaf extract, D: Rifamycin, E: Amoxycylav



CONCLUSION

The ability of bacteria to develop resistant to antimicrobial agents has become a significant problem in the treatment and control of bacterial infectious diseases. However, our results of this experiment also proved that soxhlet leaf extracts of *Cassia alata* in hydro-alcohol solvents showed good antibacterial activity against the tested pathogenic bacteria species causing wounds and urine infections. This revealed that these extracts contained compounds which were able to inhibit the growth of bacteria. Based on the traditional knowledge, this study investigated the antimicrobial activity of leaf extract by soxhlet method from *Cassia alata* aiming to add value for the local medicinal knowledge of the plant and better efficacy than those reported before and found suitable drug than low spectrum antibiotics like penicillin, amoxicillin or amoxycylav.

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