



Microbiology of Skin Disease and its Control through Herbal Drugs

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ABSTRACT

In the present study the human pathogenic organisms were isolated from skin infected person. The bacterial strains such as *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and fungal strains such as *Aspergillus niger*, *Candida albicans* and *Trichoderma sp* were used as a test pathogen. For antimicrobial activity assay *Cassia alata* was selected. Totally eight microbes were isolated from skin infected person. Among the microorganism isolated, bacteria was maximum number than fungi. Among the bacterial strains tested, the maximum susceptibility was observed in *Pseudomonas aeruginosa*, (21 mm) to *Cassia alata* extracts. Among the fungal strains tested, *A. niger* (5 mm) was inhibited maximum than other fungal strain

Keywords: Skin Disease, *Cassia alata*, antimicrobial activity, *Pseudomonas aeruginosa*, Bacterial strains.

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INTRODUCTION

The study of natural materials as potential sources of novel antibacterial agents has attracted a lot of attention throughout the last 20 years. Various extracts from conventionally used therapeutic herbs have been investigated. Some natural products have been approved as new antibacterial drugs, but there is an urgent need to identify the novel substances active towards pathogens with high resistance (Recio *et al*, 1989).

Antibacterial resistance is a worldwide issue that is becoming more challenge. The need for new principles is highlighted by the global rise in the isolation of microorganisms less sensitive to conventional antibiotics and the recovery of more resistant isolates following antibacterial therapy. Plants are frequently used in Indian traditional medicine to treat viral disorders, burns, and dermatophytes. Plants and plant products have been used extensively throughout history to treat medical problems. Numerous studies have been carried out to extract various natural products for screening antimicrobial activity but attention has not been focused intensively on studying the combinations of these products for their antimicrobial activity (Cragg *et al*, 1997).

In view of the above-mentioned facts, attempts were made in the present study to find out the antimicrobial activity of the crude extracts of *Cassia alata* against six species of human pathogenic microorganisms.



Fig 1: Cassia alata

MATERIALS AND METHODS

Sample collection

For the present study microorganisms was isolated from the skin infected person. Using sterile cotton swaps, grab a sample of pus and transfer it in a leak-proof Amies transport medium-sized container's contents. Before inserting in to the container, made a

smear of the pus on a clean slide and allow it to dry. Then labeled the sample and brought to the laboratory.

Culturing of microbes

Inoculated the specimen on blood agar (bacteria) and potato dextrose agar (Fungi) medium and it allowed overnight at 35-37^o C. Then the isolated bacterial colony was subjected to various biochemical test for species identification.

Identification of microbes

The fungi were identified by using standard manuals, such as manual of soil fungi (Gilman,1957). The physiological and biochemical tests were conducted following the methods (Beynon and Josey,1980) respectively, as described by (James and Natalie,1999) to identify the bacteria.

Preparation of leaf powder

For the present study, the medicinal plant *Cassia alata* L. belongs to family of *Fabaceae* was collected from wild medicinal valuable plants in and around area of Pattukkottai, Tamil Nadu, India. The plant was identified with the help of flora of presidency, Tamil Nadu (Lewis *et.al*, 1995).

Preparation of plant extract

The *Cassia alata* was collected washed, cut into small pieces and dried at room temperature (39°C) for two weeks and made into powder by using mixture for further analysis. The process of extracting secondary metabolites from plant matter referred to as extraction. There are essentially two kinds: heat extraction and cold extraction. Compared to cold extraction, heat extraction offers a few advantages, such as uniformity in time and lack of microbial contamination. A device known as a soxhlet was used to extract heat. Within the thimble of a soxhlet device, 100g of the plant leaf powder was placed. The plant powder towards solvent ratio was kept constant at 1:4.

Ethanol extract

The mark root the ethanol extraction was dried and extracted with 100ml of ethanol (59.5-61.5°C) by continuous hot percolation, until the extraction was completed. After the completion of extraction, the extract was filtered and the solvent was removed by distillation under reduced pressure. A dark green colored residue was obtained. The green leaves of *Cassia alata* were shade-dried at room temperature followed by being ground into a powder using an electric blender in order to prepare the extracts. 200 milliliters of ethanol were used to soak separately 50 grams of the powdered and dry ingredients for 48 hours. Extracts were concentrated under reduced pressure. The condensed products were weighed and kept at 4°C prior to test. The unidentified fractions were thus separated and tested for antibacterial and antifungal activities.

Methodology

Using the agar diffusion technique outlined by Collins and Lyne (1970), the antimicrobial activity of the extracts and fractions was determined. The extracts and fractions were produced at different quantities and evaluated against a test pathogen. The zones of inhibition were observed after the plates were incubated for 24 hours at 37°C.

RESULTS AND DISCUSSION

The plant extract of *Cassia alata* used for the present work was choosing on the basis of their medicinal values. The natural plant parts are having a wide range of medicinal properties like antiperiodic, antiphlogistic, diaphoretic, diuretic, emollient, febrifuge, narcotic, purgative and sedative. The leaves, stems and its roots are used topically as a poultice, especially in the treating of malignant sores, boils, leucoderma and wounds (Cowan, 1999).

Table 1: Biochemical characteristics of isolated bacteria

S. No	Biochemical Test	S. aureus	S. pyogens	P. auroginosa	E. coli	Klebsiella spp
1	Mac Conkey agar test	+	+	-	+	+
2	Indolent test	-	-	+	-	-
3	Methyl red test	+	+	-	-	-
4	Voges Proskauer test	+	+	+	+	+
5	Citrate utilization test	-	-	+	-	-
6	Starch hydrolysis test	+	+	-	+	+
7	Urea hydrolysis test	-	-	+	+	+
8	Nitrate reduction test	-	-	-	-	-
9	H ₂ S production test	+	+	-	-	-
10	Cytochrome oxidase test	+	+	-	+	+
11	Catalase test	-	-	-	-	-

In this study ethanol extract were tested for their antimicrobial activity against some human bacterial pathogen such as *Streptococcus mutans*, *Bacillus subtilis* and *Staphylococcus aureus*. These results are consistent

with previous reports on related plants regarding Gram-positive bacteria (Adwan *et.al*, 1998). The resistance of Gram-negative bacteria (*Staphylococcus aureus*) to plant extracts was not unexpected as in general, this class of

bacteria is more resistant than Gram-positive bacteria. Such resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism (Khan *et.al*, 2001).

Petrol, dichloromethane, and ethyl acetate have been employed to separate the methanol extracts of the leaves, stem, and root barks of *Castanopsis acuminatissima*. Despite the broad spectrum of antibacterial activity demonstrated by all of the crude methanol extracts and the resulting fractions, the activity was often reduced upon separation. None of the studied molds were resistant to them (Somchit *et al.*, 2010). This

investigation revealed that the alcoholic extract of *C. alata* showed a wide range of antibacterial activity.

Ethanol extract from leaves of *Cassia alata* was shown to exhibit antibacterial activity against *S. aureus* (inhibition zones 11 mm, respectively).

B. subtilis showed resistance to all types of extracts. Based on the current findings, it can be concluded that this plant has antimicrobial activity, which is as potent as standard antimicrobial drugs against certain microorganisms (Ogunti and Elujoba, 1993).

Table. 2: Effect of ethanol extract on fungal strain

S. No	Test Organism	Zone of inhibition (mm)
1	<i>A. niger</i>	5
2	<i>C. albicans</i>	3.5
3	<i>Trichoderma</i>	2.4

Table. 3: Effect of ethanol extract on bacterial strain

S. No	Test Organism	Zone of inhibition (mm)
1	<i>Streptococcus pyogenes</i>	21
2	<i>Staphylococcus aureus</i>	14
3	<i>Pseudomonas aeruginosa</i>	11

The six organisms evaluated in this study showed growth inhibition in response to the ethanol extracts. According to Villasenor *et al.* (2002), *Cassia alata* may also contain anthraquinone, which is the main laxative component of many plants used as purgatives. The primary component of *Cassia alata* leaf extract, flavonoid glycoside, is not shown to have any indication of providing antifungal action based on a literature search. Subsequent to fractionation with petroleum spirit, dichloromethane, and ethyl acetate, it was demonstrated that the ethanol extracts of *Cassia alata*'s leaves, flowers, stem, and root exhibited a wide range of antibacterial efficiency. According to Khan *et al.* (2001), the flower extract's dichloromethane fraction found to be the most efficient. The methanolic fraction of the leaves has been demonstrated in a recent review to exhibit no effect against *Trichophyton mentagrophytes* but to be efficacious against *Candida albicans* at a concentration of 50 mg/ml. The antibacterial properties of an extract from *Cassia alata* leaf have been documented much earlier (Palanisamy and Nagarajan, 1990).

Using the agar diffusion technique, the antibacterial activity of alcoholic extract was investigated in this work against a variety of Gram-positive and -negative bacteria as well as yeast species. Antibacterial activity was demonstrated especially against Gram-positive bacteria including multiresistant *staphylococcus* strains. The greatest activity was exhibited by the ethanol extracts then compared to another extracts. In similar reports the greatest activity was exhibited by the ethanolic extracts of *Boswellia elongata*, *Boswellia ameero*, *Buxushildebrandtii*, *Commiphora parvifolia*,

Jatrophaunicostata, *Kalanchoefarinacea*, *Pulicaria stephanocarpa*, *Punicaprotunica*, with *aniaadunensis* and with *Aniaribeckii*. Only the methanolic extract of *Buxushildebrandtii* displayed significant antifungal activity (Ramesh *et.al*, 2001).

European herbal remedies likely have higher concentrations of tanning agents, which accounts for their superior antibacterial effectiveness. On the other hand, all tested plant preparations inhibited not at all or only insufficiently the growth of the Gram-negative bacteria tested and that of *Candida albicans* (Simin, 2001). In this study growth of the Gram-negative bacteria tested against the *C. alata*.

The results of antifungal assays of *C. alata* were presented in Table 2. The extracts showed the least activity on the growth of these organisms at 70 µg /ml. However, methanol extracts suppressed the growth of *Aspergillus niger* with inhibition percentages ranging from 68 to 72%. *F. oxysporum* is a phytopathogen that causes vascular wilt and damping off in plants which could result in substantial stand reduction and yield loss. According to reports, *A. niger* was resistant to dichloromethane as well as aqueous and methanolic extracts of 14 plants that are commonly used in Paraguayan traditional medicine. However, in this study, ethanol extracts markedly inhibited the development of *A. niger*. These findings suggest that *C. alata* may be a valuable source of medicinal compounds, which might lead to new discoveries in the continuing hunt for antibacterial plants. The results corroborate the Eastern Cape's xhosas' traditional medicinal claims regarding this plant.

Conflicts of interest

Authors declare that they have no conflict of interest concerning this article.

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