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Studies on antimicrobial activities from flower extract of *Cassia alata* Linn.

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Abstract

The use of plant materials to prevent and treat infectious diseases successfully over the years has attracted the attention of scientist's worldwide. The medicinal usefulness of the plant *Cassia alata* (Linn) has been the object of many chemical and pharmacological studies. *C. alata* is an ornamental shrub or tree growing up to 12 m high and widely available in the tropics, in the grasslands and around towns and villages throughout West Africa. This tree, apart of its uses as sources of firewood and timber, has very important applications in folkloric medicine. These trees are used to treat diarrhea, dysentery and other gastrointestinal problems. The macerated juices of the young fresh leaves are used to treat eye infections and parasitic diseases. The decoction of the stem bark and roots are used to treat urinary tract infections, bronchitis and asthma. Several studies have been done to provide scientific basis for the efficacy of plants in phytomedicine. The aim of the study is to assess the antimicrobial activity and to determine the zone of inhibition of extract on some bacterial and fungal strains. In the present study, the microbial activity of methanol, chloroform and petroleum ether extracts of flowers of *Cassia alata* (Linn).were evaluated for potential antimicrobial activity against bacterial and fungal strains. The fungal isolates tested include: *Epidermatophyton floccosum*, *Microsporum gypseum* and *Trichophyton mentagrophytes* and the Bacterial isolates tested include: *Escherichia coli*. The antimicrobial activity was determined the extracts using agar well diffusion method. The flowers were shade dried and then homogenized to fine powder by a mechanical grinder. They were extracted using different solvents such as by methanol, chloroform and petroleum ether by soxhlet apparatus. The zone of inhibition was measured and tabulated.

Keywords: *Cassia alata*, *Epidermatophyton floccosum*, *Microsporum gypseum* and *Trichophyton mentagrophytes*

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Introduction

Medicinal plants constitute an effective source of both traditional and modern medicine. These plants have been shown to have genuine utility and about 80% of the rural populations depend on them as primary health care (Akinyemi, 2000). Plants have been used as sources of remedies for the treatment of many diseases since ancient times and people of all continents especially Africa have this old tradition. Despite the remarkable progress in synthetic organic medicinal products of the twentieth century, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants (Newman et al., 2000). However, plants used in traditional medicine are still understudied (Kirby, 1996; Hostellmann and Marston, 2002). In developing countries where medicines are quite expensive, investigation on antimicrobial activities from ethnomedicinal plants may still be needed. It is on this basis that researchers keep on working on medicinal plants in order to develop the best medicines for physiological uses (Usman and Osuji, 2007). In developing countries, notably in West Africa, new drugs are not often affordable. Thus, up to 80% of the population uses medicinal plants as remedies (Hostellmann and Marston, 2002). As a result of the indiscriminate use of antimicrobial drugs in the treatment of infectious diseases, microorganisms have developed resistance to many antibiotics (Cowan, 1999). There is need to develop alternative antimicrobial drugs. One approach is to screen local medicinal plants which represent a rich source of novel antimicrobial agents. In Africa today, 80% of the

population uses traditional medicine in primary health care (WHO, 2006). Many African plans are used in medicine as antimicrobial agents but only few have documented. However, in spite of vast improved health and longevity in the United State and Europe, millions of their people are turning back to traditional herbal medicine in order to prevent or treat much illness and to circumvent resistance of many human pathogens to conventional (antibiotics), some of which have side effects like hypersensitivity and immunosuppression.

In Nigeria, traditional medical practitioners use a variety of herbal preparation to reat different microbial diseases. For example, the Ebira tribes of Kogi state, Nigeria use the fruits of solanum melangena for weight loss allowed as dietary delicacy (Bello et al., 2005). The use of herbal medicine predates the introduction of antibiotics predates social economic and religious barriers (Akinyemi et al., 2000). The use of medicinal herb in the treatment and prevention of diseases is attracting attention by scientists' worldwide (Upadhyay et al., 2010). World Health Organization corroborated this in its quest to bring primary health care to the people. The plant kingdom has for long time served prolific source of helpful drugs, food, additives, flavouring agents, colorants binders and lubricants etc, as a matter of facts, it was estimated that about 25% of all prescribed medicines today are substances derived from plants (Bello et al., 2005). For control of microbial infections and diseases, various synthetic drugs and chemical formulations have been used. But due it their indiscriminate use, microbes have developed wide resistance against these synthetic drugs such as broad-spectrum antibiotics.

This resistance was developed after induction of new enzymes system in microbes which not only simplify drugs but also enhance drug threshold level in microbes. Therefore, to combat the problem of microbial infection and drug resistance new alternative of synthetic drugs have been explored, though antimicrobial activities of so many natural products have not been explored (Upadhyay et al., 2010). Many reports have shown that some *Cassia* species contain antimicrobial substances, particularly *Cassia alata* (Fuzellier et al., 1982; Caceres et al., 1991; Crockett et al., 1992; Caceres et al., 1993; Ibrahim and Osman, 1995; Agarkar and Jadge, 1999; Khan et al., 2001; Villasenor et al., 2002; Somchit et al., 2003). *Cassia* is a native plant in Southeast Asia, Africa, Northern Australia and Latin America (Parsons and Cuthbertson, 1992). In this study, we focused on the *in vitro* antimicrobial activity testing of methanol, chloroform and petroleum ether extracts of the flower of the Cassia alata against bacterial and fungal strains such as *Escherichia coli*, *Epidermatophyton floccosum, Microsporum gypseum* and *Trichophyton mentagrophytes*.

Materials and methods

Plant material

The *Cassia alata* Linn. Flowers were procured in July 2011 from the premises of Chennai. The plant and plant material were identified and authenticated by Prof.P.Jayaraman, Ph.D. Plant Anatomy Research Centre, Tambaram, Chennai.

Extraction of crude drug

The plant materials were shade dried and then powdered with the help of a blender. 50 g of the powder was filled in the thimble and extracted successively with petroleum ether, chloroform and methanol using a Soxhlet extractor for 48 h. All the extracts were concentrated using rotary evaporator. All the extracts were subjected to antibacterial and antifungal activity assay.

Microbial cultures

Antimicrobial activity of flowers extract was investigated against one bacterial isolates viz. *Escherichia coli* and three fungal strains viz. *Epidermatophyton flocossum, Microsporum gypseum* and *Trichophyton mentagrophytes*.

Antimicrobial activity

The antimicrobial assay was performed by two methods viz. agar disc diffusion method (Rios et al., 1988;

Table1. Antimicrobial properties of methanol, chloroform and petroleum ether extract of *Cassia alata* flower

Microorganisms	Solvents														
	Methanol µg/ml					Chloroform µg/ml					Petroleum ether µg/ml				
	100	75	50	25	C	100	75	50	25	C	100	75	50	25	C
	Zone of inhibition (mm)														
E.coli	20	20	15	13	-	15	-	-	-	-	-	-	-	-	-
E.floccosum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M.gypseum	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T.mentagrophytes	10	09	-	-	-	10	09	-	-	-	19	17	-	-	-

Parekh et al., 2005) and agar well diffusion method (Bauer et al., 1966). The Petri dishes were cleaned, dried, sterilized and filled with nutrient agar medium with uniform thickness. After solidifying, the plates were inoculated with the bacterial fungal strains such *Escherichia coli*, *Epidermatophyton floccosum*, *Microsporum gypseum* and *Trichophyton mentagrophytes*. The extracts were dissolved in solvents at a concentration of 25, 50, 75 and 100 µg/ml. All the Petri dishes were incubated at 37ºC for 24 hrs. The assessment of antibacterial activity was based on the measurement of diameter of inhibition zone formed and the zone was measured.

Results

The antibacterial activity of *Cassia alata* flower extract was assayed *in vitro* by agar disc diffusion and agar well diffusion method against bacterial and fungal strains. Table 1 summarizes the microbial growth inhibition of petroleum ether, chloroform and methanol extracts of *Cassia alata* flower were studied in different concentrations (25, 50, 75 and 100 µg/ml) against one bacterial strain (*E.coli*) and three fungal strain (*Epidermatophyton flocossum*, *Microsporum gypseum* and *Trichophyton mentagrophytes*). In the present study, antibacterial and antifungal activity of the extracts increased linearly with increase in concentration of extracts (µg/ml) Petroleum ether, Chloroform and methanol extracts obtained from Cassia alata flower showed mild to strong activity against most of the tested bacteria and fungi. Of the three extracts, methanol extract displayed activity *E.coli* (20, 15, 13) shows excellent result as compared to *T.men* (10 and 09) and *M. gypseum* (16). There is no inhibition in *E. flocossum*. Chloroform extract showed activity T.men (10, 09) shows good result as compared to *E.coli* (1.5) and there is no inhibition in *M. gypseum* and *E. flocossum*. Petroleum ether extract showed activity *T.men* (19, 07) shows mild activity as compare to *E. flocossum*, *M. gypseum* and *E. coli*. On overall consideration, of petroleum ether and chloroform extract were not so enough as those of methanol. It is also evident that the extracts of cassia alata flower were found to be mild active against most of the bacterial and fungal strains (Table 1).

Discussion

The results obtained from this study show that *Cassia alata* extracts are inhibitory towards microorganisms (*E.coli*, *Epidermatophyton flocossum*, *Microsporum gypseum* and *Trichophyton mentagrophytes*). These findings confirm the traditional therapeutic claims for these herbs to treat ringworm and skin diseases. Previous reports of antimicrobial activity against human pathogens have been widely carried out for *C. alata* (Palanichamy and Nagarajan, 1990; Ibrahim and Osman, 1995) found that 5% aqueous extract from leaves of *C. alata* and some of its components, rhein, emodol, 4,5-dihydroxy-1 hydroxymethylanthrone and 4,5-dihydroxy-2-hydroxymethylanthraquinone, had antifungal activityagainst some dermatophytes and yeast. *C. alata* leaves extracted with petroleum ether followed by hot 85% ethanol under reflux and tested for its antifungal activity

against *Candida albicans, Aspergillus fumigatus, Aspergillus flavus, Mucor* spp., *Rhizopus* spp. and dermatophytes; *Trichophyton mentagrophytes, T. rubrum* and *M. gypseum*. They reported that 20% w/v crude extract did not show any significant activity against the contaminant fungi, whereas 2.5 and 3% crude extract completely inhibited the growth of dermatophytes. Most of the extracts tested, either extracted by aqueous, alcohol, hexane, petroleum ether or ethyl acetate exhibited anti dermatophytic activity. In this study methanol, chloroform and petroleum ether was used as the solvent. *C. alata* methanol extract was the most active solvent against (*E.coli*, *Microsporum gypseum* and *Trichophyton mentagrophytes*). Although, of the three extracts, petroleum ether and chloroform extracts is inactive against most of the bacteria and fungal strains. So we got good results in zone of inhibition.

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