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Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2010, 2(4):304-308

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Antimicrobial Activity of *Cassia didymobotrya* and *Phlogacanthus thyrsiflorus*

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ABSTRACT

The in vitro antimicrobial activity of Cassia didymobotrya and Phlogacanthus thyrsiflorus leaf extracts were studied against selected bacteria and fungi following Agar disc diffusion method. Leaves were extracted using distilled water, methanol and ethyl acetate. Three different concentrations were applied to the disc (100µg, 250µg and 500µg/disc) for each extracts. While the aqueous extracts of the selected plants leaves showed mild to moderately effective, the methanol and ethyl acetate extracts were more efficient compared to the aqueous extract. The inhibition zone diameter was seen to increase with the concentration. The results were compared with results obtained using standard antibiotics Kanamycin and Fluconazole which serve as a reference for inhibition zone diameter.

Keywords: *Cassia didymobotrya*, *Phlogacanthus thyrsiflorus*, Antimicrobial activity, Agar disc diffusion method.

INTRODUCTION

Medicinal plants, since time immemorial, have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies and healthcare preparations, as those described in ancient text such as the Vedas and Bible, and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties [1].

Today there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the

current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects [2].

An increasing incidence of bacterial and fungal infections can be seen during the past several years due to a growth in immune compromised population. With the resistance to antibiotics and the toxicity during prolonged treatment with several antimicrobial drugs [3] has been the reason for an extended search of newer drugs to treat opportunistic microbial infection [4]. At present, synthetic antifungal/ antibacterial drugs widely used sometimes causes toxicity and adverse drug reactions. Furthermore herbal medicines and supplementation are considered less toxic than the synthetic compounds [5],[6],[7].

Cassia didymobotrya belongs to the family Caesalpiniaceae. It is also known as Popcorn Cassia, Peanut Butter Senna. Popcorn cassia is a shrub or small tree producing golden yellow flowers, opening from buds which are enclosed in greenish black bracts. The plant looks quite similar to Candle bush, and grows up to 0.5-5m tall. The flowers distinctly small of peanut butter, which inspired the common names. It is native to Africa, naturalized wide across the world. It can be seen round the year. In India, it was introduced as a green manure and as a garden plant and is very common along the roadside of Imphal valley. This elegant shrub has arching branches with compound leaves, 10-15 cm long, with leaflets in 8-18 pairs. It blooms beautiful large clusters of bright yellow flowers. Fruit is a flat, 9-16 seeded pod, linear-oblong 7-12 cm x 1.5-2.5 cm, glabrescent, short beaked, dehiscent or indehiscent when dry, depressed between the seeds, sutures raised, blackish-brown. It is widely used a medicinal plant especially in Africa. The decoction or infusion from the leaves, stems and roots is drunk as a laxative and purgative for the treatment of abdominal pains, while in large quantities it is taken as an emetic. It is also taken to expel intestinal worms and to treat ringworms. The powder of the root or leaf mixed in water or a decoction of the fresh parts is taken to treat abscesses of the skeletal muscles and venereal disease. The plant is also indicated for the treatment of fungal and bacterial infections, hypertension, haemorrhoids, sickle-cell anaemia and a range of women's diseases, such as inflammation of the fallopian tubes, fibroids and backache, to stimulate lactation and to induce uterine contractions and abortion [8],[9].

Phlogacanthus thyrsiflorus is a gregarious shrub. It belongs to the family Acanthaceae. This plant has long dark or blackish red tubular flowers, appearing in upright spikes at the end of the branches and grows up to a height of 3 m. Leaves are oblong-ovate deep green, 15-20 cm long and 6-8 cm broad. It is commonly called *Nongmakha* in Manipuri and *Kala adusa* in Hindi. The plants can be seen growing mostly during Dec-April and is distributed throughout the tropics and in the entire North East Region of India. The whole plant is extensively used for its great medicinal value. Leaf juice is used in cough, phlegm, asthma, bronchial disorders, jaundice, diarrhoea, dysentery, tuberculosis, malarial fever, as an anti-allergic and in rheumatism while the ethanol extract is reported to show analgesic activity. Flower is edible and is used as vegetable. Medicinal salt extracted from the ash of whole plant is used in cases of indigestion, gastritis, pharyngitis, cough, asthma and checked acidity. The paste of root is used in case of chronic leucorrhoea. The smoke of leaf is used during asthma attack in the form of a cigarette [10-12].

EXPERIMENTAL SECTION

The fresh leaves of *Cassia didymobotrya* and Phlogacanthus *thyrsiflorus* were collected randomly during the month of March-April, from the Imphal East and Imphal West districts of Manipur and were authenticated. The plants materials were taxonomically identified by

taxonomist and the voucher specimens have been preserved in the laboratory for future references.

Preparation of extracts: Fresh leaves were dried under shade with occasionally shifting and then powdered with a mechanical grinder and stored in air-tight containers. The dried materials were extracted using distilled water, Ethyl acetate and Methanol as the solvent system.

Aqueous Extraction: 10g of dried powder was added to 100ml of distilled water in a conical flask and boiled on slow heat for 2 hrs. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000g for 10 min. The supernatant was collected. After 6 hrs, the supernatant collected at an interval of every 2 hrs was pooled together and concentrated using a rotary evaporator. The residue obtained were collected and stored at 4 °C in airtight bottles

Methanol Extraction: 10g of dried powder was taken in 100ml of methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 hrs. After 24 hrs. the supernatant was collected and the solvent was evaporated through a rotary evaporator. The residue obtained were collected and stored at 4 °C in airtight bottles.

Ethyl Acetate: 10g of dried powder was taken in 100ml of ethyl acetate in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 hrs. After 24 hrs the supernatant was collected and the solvent was evaporated. The residue obtained were collected and stored at 4 °C in airtight bottles.

Test microorganisms: Three Gram +ve and Gram –ve bacterial and two fungal strains were used for the antimicrobial assay. They were: *Bacillus subtilis* (ATCC6633), *Staphylococcus aureus* (ATCC25923), *Micrococcus leteus* (ATCC10240), *Esherichia coli* (ATCC25922), *Enterobacter aerogenes* (ATCC13048), *Pseudomonas aeroginosa* (ATCC7853), *Aspergillus niger* (ATCC16404) *and Candida albicans* (ATCC10231). Microorganisms were obtained from the National Chemical Laboratory (NCL), Pune, India. All the bacterial strains were maintained on nutrient agar and fungal strains in potato dextrose agar at 4 °C.

Antimicrobial Assay: Antimicrobial activity of the three solutions of extracts was determined by the disc diffusion method. Suspensions of microbial strains with an optical density of McFarland 0.5 were made in distilled water. Sterile petri dishes of diameter 14 cm with 60 ml of sterile Nutrient Agar and Potato dextrose agar were seeded with appropriate microbial suspension. Sterile paper disc (Whatmann, 5mm diameter) were impregnated with the solution 100, 250 and 500 µg/disc and disc were allowed to dry and then discs were spaced on the surface of each sterile petri dishes. Negative control of discs with water, methanol and ethyl acetate were included. *Kanamycin* (30mg/disc) and *Fluconazole* (50mg/disc) were used as antibacterial and antifungal standards for comparative and control purpose. The tests were repeated three times and the mean values were represented.

RESULTS AND DISCUSSION

The antimicrobial activity of *C. didymobotrya* and *P. thyrsiflorus* leaves extracts were analysed in vitro by Agar disc diffusion method against 6 bacterial and 2 fungal species.

It was observed that the methanol and ethyl acetate extracts showed higher antibacterial activity compared to the aqueous extract. While the extracts were effective against most of the tested bacterial strains but they fail to exhibit activity against *P. aeroginosa.* Inhibition zone diameter

obtained (mm) was given in **Table-1**. From the Table, it can be observed that where activity is not seen with lower concentration, higher concentration of the extracts could exhibit the activity. It was also observed that the leaves extract exhibited antifungal activity against *A. niger* and *C. albicans.* While no activity was observed in certain cases with lower concentration, tested extracts at higher concentration exhibits comparable antifungal activity with that of the Standard drugs.

Table 1. Inhibition zone diameter (mm) of Antimicrobial activity

Bacteria	**Conc^{n}.**	***C. didymobotrya***			**Conc^{n}.**	***P. thyrsiflorus***			**Kanamycin**
	(µg)	**Water**	**Methanol**	**E.Acetate**	**(µg)**	**Water**	**Methanol**	**E. Acetate**	**30mg/disc**
*B. subtilis**	100	-	8	-	100	-	9	8	24
	250	10	10	8	250	8	10	11	
	500	13	16	12	500	10	13	14	
*S. aureus**	100	-	-	-	100	-	-	9	23
	250	-	-	8	250	8	9	12	
	500	10	12	11	500	11	13	14	
*M. leteus**	100	-	9	-	100	-	8	10	21
	250	8	10	12	250	10	10	13	
	500	10	15	13	500	12	13	15	
*E. coli***	100	-	-	-	100	-	7	9	22
	250	-	9	8	250	11	8	10	
	500	11	12	10	500	12	12	13	
*E. aerogenes***	100	-	8	-	100	-	9	8	22
	250	9	12	11	250	12	10	12	
	500	13	15	12	500	13	13	16	
*P. aeroginosa***	100	-	-	-	100	-	-	-	23
	250	-	-	-	250	-	-	-	
	500	-	-	-	500	-	-	-	
Fungi									**Fluconazole 50mg/disc**
A. niger	100	-	-	-	100	10	12	-	15
	250	8	12	8	250	8	11	12	
	500	12	17	15	500	13	17	16	
C. albicans	100	-	8	10	100	-	-	9	14
	250	9	12	13	250	11	15	10	
	500	13	15	15	500	15	16	17	

* Gram positive bacteria** Gram negative bacteria

It can be seen that the extracts showed a broad spectrum of activity against most of the microbial strain at the tested concentration of 100-500µg/ml. Kanamycin (30mg/disc) and Fluconazole (50mg/disc) were used as positive control for bacteria and fungi.

It is evident from the result, that the extract of the selected plant shows antimicrobial activity in a dose dependent manner. The activity increases with the increase in the concentration of the extracts. As reported earlier secondary metabolites like flavonoids, saponins are likely responsible for the antimicrobial activity of plants [13],[14],[15]. The antimicrobial activities of these plants may be also due to the presence of these active components in their leaves. Thus, it can be concluded that the crude extracts of plants can be used as drugs against those organisms which are sensitive to it. Here, the development of antimicrobials from higher plants appears to be rewarding. However, before use in human isolation of pure compounds, their toxicological studies and clinical trials in animal model should be carried out.

Acknowledgement
The authors are thankful to the Department of Biotechnology, Government of India, for its Financial Support which makes the work a success.

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