JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2009. Vol. **3**(2), p. 793-796

Antifungal Activity of *Calotropis procera* (Ait.) R. Br. flowers

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(Received: 08 May 2009; accepted: 04 June 2009)

Extracts drawn from dried and powdered flowers, of *Calotropis procera* with some organic solvents and aqueous were tested for antimicrobial effect against the fungi, *Cladosporium* sp., *Mucor* sp., *Aspergillus flavus* and *Fusarium oxysporum*. Methanol was the best solvent to extract antimicrobial compounds from flowers and was superior to chloroform, water and petroleum ether. The strongest inhibitory effect of the extracts was observed against *Cladosporium sp*., *Mucor sp*. The weakest effect was against *Fusarium oxysporum*. Results clearly indicate that the flowers of *Calotropis procera* is a promising source of antimicrobial compounds.

Key words: *Calotropis procera*, Asclepiadaceae, Antifungal activity, Bioactive compounds.

Plants have been formed the basis of natural pesticides, that make excellent leads for new pesticide development[1]. The potential of higher plants as a source of new drugs is still largely unexplored. Hence, last decade witnessed an increase in the investigation on plants as a source of new biomolecules for human disease management[2]. Traditionally plants have been well exploited by man for the treatment of human diseases, Ayurveda is a good example, but not much information is available on the exploitation of plant wealth for the management of plant diseases, especially against phytopathogenic fungi.

Fungi cause severe damage to stored food commodities. Among different species of fungi *Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp. are associated with heavy loss of grains, fruits, vegetables and other plant products during pickling, transit and storage rendering them unfit for human consumption by producing mycotoxins and affecting their nutritive value[3-5]. Many seed borne fungi, which cause severe damage to stored food commodities, were generally managed by

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synthetic chemicals, which were considered both efficient and effective. The continuous use of these synthetic fungicides started unraveling non biodegradability and known to have residual toxicity to cause pollution[6]. Pesticide pollution of soil and water bodies is well documented[7]. Hence in recent time application of plant metabolites for plant disease management has become important viable component of Integrated Pest Management, as plant metabolites are eco-friendly.

The species of Calotropis are commonly known as the Milkweed belonging to the family Asclepiadaceae. Calotropis is regarded as useful medicinal plant and used in folk medicine[8-10]. Although the latex of *Calotropis procera* has been extensively studied and found responsible for cytotoxic, anti coagulant, anti- inflammatory, abortifacient activities[11]. The medicinal properties of the flowers of this plant are not well explored. Therefore, the present study was designed to evaluate the effect of the flower extract of *C. procera* on pathogenic fungal strains.

MATERIAL AND METHODS

Collection and Identification of plant material

The fresh flowers of *C. procera* were collected from in and around areas of Coimbatore, Tamilnadu. The plants were identified and classified by Prof. Mohanan (taxonomist) of Botanical Survey of India, TNAU, Coimbatore. The plant samples were deposited in herbarium.

Preparation of extracts

The flowers were washed thoroughly under running tap water and 70% alcohol to free them from dust and other contaminated particles. The flowers were shade dried, powdered and extracted (30 g) sucessesively with petroleum ether (150 ml), chloroform (130 ml), methanol (120 ml) in a soxhlet extraction for 20 hr. the extract was evaporated to dryness at 37ºC. The aqueous extract was prepared by soaking 30g of powder in 200ml of distilled water. After 24 hours elapsed with interval stirring, the mixture were filtered using Whatman No. 1 filter paper and the filtrate was left for dryness by evaporation using steam bath at 100ºC. The dried extracts were stored at 4ºC for further investigations.

Analysis of bioactive compounds

The various extracts were analyzed for alkaloids, tannins, phenols, steroids, glycosides, saponins, flavonoids and fixed oils were carried out by standard procedures[12,13].

Test pathogens

Fungal pathogens like *Aspergillus flavus, Fusarium oxysporum, Cladosporium* sp*, Mucor* sp were the pathogens selected for the study. The fungal species in potato dextrose agar slants and stored at 4°C.

Antifungal screening

This was carried out by disc diffusion method[14]. In this method 20ml of potato dextrose agar medium was plated in petridish with 0.2 ml of a 10^{-2} dilution of each fungal culture (10h old). The respective extracts (10mg/ml) were used to saturate the disc (Whatman no.1, 6mm) and placed on the seeded plates. The activity was determined after 72h of incubation at 30°C. The diameter of zone of inhibition produced were recorded. Each sample was used in triplicate for the determination of antifungal activity. Amphotericin B was used as control. The minimum inhibitory concentration (MIC) of methanol was also determined by above said method by using various concentrations (0.5 mg/ml, 1mg/ml, 2 mg/ml, 5 mg/ml, and 10 mg/ml) of crude extracts.

RESULTS AND DISCUSSION

The various extracts of flowers of *C. procera* had antimicrobial effects against all fungi studied. According to zone of inhibition, activity against test microorganisms showed in Table 1. In general methanolic extract exhibited stronger antifungal activity followed by chloroform and water. The petroleum ether extract exhibit no activity on the pathogens studied.

Antifungal activity

Solvent extracts

The greatest inhibition of *cladosporium* sp*.,* (15mm) and lowest inhibition against *Fusarium oxysporum*, (10mm) was observed with methanol followed by chloroform (Table 1). Rest of the test pathogens exhibited mild zones of inhibition. The MIC results are shown in Table 2. Antifungal agents with low activity against an organism have high MIC value while highly active antifungal agents have low MIC value.

Table 1. Antifungal activity of methanolic extract of *C.procera* flowers

Extract(1mg/ml)	Zone of inhibition (mm)			
	Cladosporium sp.,	*Mucor* sp.,	*Aspergillus flavus*	*Fusarium oxysporum*
Petroleum ether	-	-	-	-
Choloroform	10	11	9	9
Methanol	15	12	11	10
Aqeuous	9	9	8	7
Amphotericin B B	14	12	18	14

Aqueous extract

The aqueous extract has shown moderate activity on all the pathogens studied likely *Cladosporium* sp. (9mm), *Mucor* sp. (7mm), *Aspergillus flavus* (8mm), *Fusarium oxysporum* (7mm).

Phytochemical analysis

Phytochemical analysis of methanol extract revealed the presence of carbohydrates and cardiac glycosides, proteins and amino acids, alkaloids, phenolic compounds and tannin. Oils, gum and mucilage were found absent in methanol extract (Table 3).

The higher plants may play an important role in controlling many plant diseases, including those of other higher plants[20]. Plants produce natural chemicals that are possible sources of non-

Table 2. MIC results of methanolic extract

Extract	Pathogens	MIC value in mg/ml
Methanol	*Cladosporium* sp.	0.2
	Mucor sp.	0.3
	Aspergillus flavus	0.5
	Fusarium oxysporum	1.0

Table 3. Bioactive compounds of *C.procera* flowers

Bioactive Compound	Petroleum Ether	Chloroform	Methanol
Alkaloids	+	+	+
Tannins	+	+	++
Phenols	-	-	+
Cardiac Glycosides	++	++	+++
Flavanoids	+++	+++	+++
Saponins	+	+	++
Steroids	+++	+++	+++
Fixed oil	–	–	–

In this first study on the antimicrobial activity of *C. procera* against plant pathogenic fungi, flower extracts proved a promising source of antimicrobial compounds. Variations in the antifungal effectiveness of different extracts against different organisms were most likely due to differences in the nature of the inhibitory materials they contained[15]. Results from our phytochemical analysis (Table 3) suggest that the presence of biologically active compounds like carbohydrates and cardiac glycosides, proteins and amino acids, alkaloids, phenol and tannins in the plant extracts could be related to the antifungal activity [16,17]. The mechanism(s) of action of the constituents of *C. procera* might be due to the inhibition of fungal cell wall, protein and amino acid, sphingolipid biosynthesis and electron transport chain[18,19].

phytotoxic, systemic and readily biodegradable alternative pesticides[21] and the extracts of many plant species have antifungal activity[22,23]. This is the first paper reporting the antifungal activity of flowers of *C. procera* crude extracts.

The finding suggest that *C. procera* flowers is a potential source of compounds that are effective against many fungi (Table 2). Further studies on *C. procera* flowers are recommended to identify the antifungal compounds. Identifying such compounds was beyond he scope and purpose of this study.

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