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Full Length Article

Antifungal Activity of Leaf and Latex Extracts of *Calotropis procera* (Ait.) against Dominant Seed-Borne Storage Fungi of Some Oil Seeds

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ABSTRACT

In present study, aqueous and ethanol extracts of leaf & latex of *Calotropis procera* (Ait.) was tested for their antifungal activity against dominant storage seed-borne fungi of some oil seeds such as groundnut, soybean, sunflower and mustard. The antifungal effect of ethanol and aqueous extracts of leaf & latex of *Calotropis procera* (Ait.) against ten seed-borne dominant fungi viz., *Cuvularia lunata, Alternaria alternata, Rhizoctonia solani, Fusarium solani, Penicillium chrysogenum, Aspergillus niger, A.* f*lavus, A. terrus A. fumigatus, and Rhizopus sp.,*were determined using agar well diffusion methods. The results revealed that ethanol was the best extractive solvent for antimicrobial properties of latex of *C. procera* followed by aqueous.

Key Words: Antifungal activity, latex, *Calotropis procera,* Carbendazim.

INTRODUCTION

In recent years interest is growing to evaluate plants possessing antimicrobial activity (Clark *et al*., 1993) Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey, 1999). The abundance of plants on the earth's surfaces has led to an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agents (Bonjar *et al*., 2004). Biologically active compounds present in the medicinal plants have always been of great interest to scientists working in this field. Chemical fungicides cause serious environmental problems (Anon, 2005). The toxic effect of synthetic chemicals can be overcome by search of new pesticides, which are eco-friendly and effective. Search of natural fungicides from the plant sources would definitely be a better alternative to these hazardous chemicals (Mishra *et al*., 2009). Various plant extracts have played significant role in the inhibition of seed-borne pathogens (Mansilla and Palenzuela, 1999; Neerman, 2003). *Calotropis procera* (Ait) R.Br. (*Asclepiadaceae*) is a plant growing widely throughout the tropical and subtropical regions of Asia and Africa and Middle East (Singhal *et al.,* 2009). This plant is popularly known due to the abundance of latex in its green parts which is easily collected when the plant is wounded. Such a fact reinforces the idea that this milky latex is accumulated as a defense strategy against insects, viruses and fungi (Deepak, 1995). Latex is a source of various biologically active compounds, including glycosides, tannins and many proteins, among others. (Wititsuwannakul *et al*., 2002 and Dubey *et al*., 2003).

The plant is reported for analgesic activity, anti-inflammatory, and hepatoprotective effects (Dewan *et al.,* 2000; Alencar *et al.,* 2004; Padhya *et al.,* 2007). However, little known about the antimicrobial activities of *Calotropis procera* except for their activities against a small range of microorganisms (Jain *et al.,* 1996; Kareem *et al.,* 2001). Recently, an antifungal protein was purified from the latex of *Calotropis procera* (Freita *et al*., 2011). In the present study, the antifungal activity of solvent extraction of leaf and latex of *Calotropis procera* was investigated and compared with standard fungicide.

MATERIAL AND METHODS

Plant material and latex collection-

The healthy and mature leaves of *C. procera* were collected from Nanded region. Their identification was confirmed using the 'Flora of Marathwada' (Naik, 1998). Fresh leaves were washed with sterile distilled water, air dried and then homogenized to fine powder and stored in airtight bottles. The fresh latex of *C. procera* was aseptically collected in clean glass tubes from the aerial parts of the healthy plant early in the morning. Latex sample was brought to the laboratory. The latex sample was oven dried at 42°C. Dried latex was powdered using mortar and pestle. Chemical fungicide Carbendazim was collected from authorized agrochemical shops from local market of Nanded.

Extraction:

The extraction was carried out using the solvent ethanol and aqueous. 10gm of dried leaves and latex was weighed accurately and dissolved in 100 ml of appropriate solvent ethanol in an air tight cork bottle. The suspended solutions were kept in rotary shaker for 24 hours and the supernatant was concentrated by drying while aqueous extract was dried using water bath. Dried extract was used for bioassays and stored at 4ºC until use (Parekh, 2007).

Test microorganisms:

The latex extracts of *Calotropis procera* was tested for antifungal activity against dominant storage seed-borne fungi of some oil seeds viz. *Cuvularia lunata, Alternaria altrenata, Rhizoctonia solani, Fusarium solani, Penicillium chrysogenum, Aspergillus niger, A.* fl*avus, A. terrus A. fumigatus, and Rhizopus stolonifer..*

Determination of antifungal activity-

Antifungal activity of the above mentioned extracts was determined, using agar-well diffusion method. (Perez *et al.,* 1990). The extracts were dissolved in DMSO to obtain final concentration of 100 mg/ml. 100 µl of test compound was introduced into the well and plates were incubated at 28°C ± 2°C for 48 hours. Dimethyl sulfoxide (DMSO) was used as a negative control. Carbendazim is used as standard fungicide at 50 ppm.

Table: **Antifungal activity of *Calotropis procera* leaves extract against seed- borne fungi**.

Sr. No.	Test organism	Zone of inhibition (mm)				
		Leaf extract		Latex extract		Carbendazim (50 ppm)
		Aqueous	Ethanol	Aqueous	Ethanol	
1.	*Alternaria alternata*	09	14	10	15	25
2.	*Aspergillus flavus*	07	09	10	13	28
3.	*A. fumigatus*	08	12	09	12	20
4.	*A. niger*	08	10	11	15	30
5	*A.terrus*	09	11	10	13	29
6.	*Cuvularia lunata*	08	11	12	16	32
7.	*Fusarium solani*	10	13	12	20	32
8.	*Rhizoctonia solani*	10	13	09	16	30
9.	*Penicillium chrysogenum*	12	15	13	18	35
10.	*Rhizopus stolonifer.*	-	0	-	-	22

RESULTS AND DISCUSSION

Plant derived compounds (phytochemicals) have been attracting much interest as natural alternatives to synthetic compounds. These natural products provide clues to synthesize new structural types of antimicrobial and antifungal chemicals that are relatively safe to man and it can help to meet expensive and limited supply of synthetic chemicals. Latex has antimicrobial properties against many species (Thomas *et al.,* 1989). A recent research indicated that many chemical compound, which present in rich amounts in several plants exhibited antioxidant, antifungal, antibacterial and anti-inflammatory properties (Shalini and Srivastava, 2009). The results obtained showed that both the leaves and latex of *C. procera* have fungicidal effects on test organisms. The results shown in the table indicated that, ethanol was the best solvent for extracting antimicrobial substances from this plant as compared to aqueous. The ethanolic extracts of both leaf and latex *C .procera* showed inhibition of growth in the test fungi with the widest zone of inhibition as compared to aqueous except *R.stolonifer*. There were significant differences in their activities depending on the microorganism tested and the solvent used with diameters of inhibition zones ranged between 07mm to 20mm.

Ethanolic & aqueous leaf extract showed widest zone in inhibition (15 & 12mm respectively) against *Penicillium chrysogenum,* whereas lowest zone of inhibition showed against *A.flavus.* (09mm & 07mm respectively) Aqueous latex extract showed widwest zone of inhibition against *Penicillium chrysogenum* and *Fusarium solani, Cuvularia lunata* (13mm & 12mm respectively) whereas lowest zone of inhibition against showed against *Rhizoctonia solani & A. fumigatus.* (09 mm) Ethanolic latex extract showed widwest zone of inhibition against *Fusarium solani & Penicillium chrysogenum.* (20mm & 18mm respectively) However, few reports are available on the antimicrobial activity of *C. procera*. Recently Kareem *et al,.* (2008) have reported that the latex of *C. procera* possess significant inhibitory effect on fungal strains. Similarly Hassan *et al.* reported that the aqueous extracts of *C. procera* stem, root and leaves have demonstrated strong inhibitory effect on the test microorganisms. The effect of *Calotropis procera* extracts agrees with the work of Nenaah *et al*., (2011) who evaluated the antimicrobial activity of extracts of leaf and latex of *Calotropis procera* and also examined the synergistic interaction between *C. procera* latex and antibiotics. The effect of *Calotropis procera* against *Fusarium solani* agrees with the work of Freitas et al. (2011) which analyzed the inhibitory effect of extracts of latex of *Calotropis procera* against *Fusarium solani.* The effect of *Calotropis procera* latex also agrees with the work of Raghvendra & Mahadevan (2011) which showed antifungal activity against *A.niger.* The effect of *Calotropis procera* extracts against seed-borne fungi agrees with the work of Abdulmoniem *et al*., (2012) who evaluated the antimicrobial activity of extracts of leaf and latex of *Calotropis procera.* Chemically, the latex of *C. procera* is composed of various classes of phytochemical compounds. Theses were extensively proved in various studies which include proteolytic enzymes, cardenolides, alkaloids, cardioactive glycoside like calactin, calotropain, proceroside, syriogenine, calotoxin and uscharin, as well as tannins, flavonoids and procerain, a stable cysteine protease (Mossa et al., 1991: Deeepak, 1995: Dubey and Jagannadham, 2003). One or more constituents of the latex, separately or in combination, may be responsible for the antimicrobial activity of *Calotropis procera* (Deeepak, 1995; Dubey and Jagannadham, 2003). Pathak & Zaidi (2013) reported that latex of *C. procera* has been found quite effective in controlling the seed-borne mycoflora of wheat. Varahalarao et al., (2010) examined biosssays for antimicrobial activities of stem, leaves and flowers of *C. procera*, the antimicrobial activities of the organic solvent extracts are tested on the various test organisms *Alternaria alternata, Aspergillus niger, A. flavus Curvlaria lunata, Penecillium expansum Rhizpctonia solani, Bipolaris bicolar.* Sharma et al., (2002) worked on screening of leaf extracts of *C. procera* for their fungicidal properties.

The results showed that, *Calotropis procera* latex possesses antifungal activity. Future research should focus on the more elucidation of the chemical constituents and their mechanism of action to facilitate efficient uses of important plant resources as anti-microbial drugs.

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