Madras Agric. J., 95 (7-12) : 386-389 July-December 2008
https://doi.org/10.29321/MAJ.10.100599

Antifungal activity analysis of *Calotropis procera*

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Abstract : *Calotropis procera* (R. Br.) is a semi woody weed plant belongs to Asclepidaceae, family commonly known as arka, madar, mudar *etc.*, The various parts of the giant milkweed tissues possess numerous medicinal properties, especially the root bark due to the presence of resin, cardenolids, steroid glucosides *etc.* To evaluate the antifungal activity of *Calotropis procera,* HPLC analysis of free fatty acid methyl ester and antifungal activity against *Fusurium, Trochosporium vesiculatwn* using the samples of methanol extract, water extract and ethyl acetate extract was carried out. Isolates of fungal pathogens *viz. Fusarium* and *T. vesiculatum* were made from freshly prepared disease samples. Dimethyl sulfoxide (DMSO) was used as control. The setups were incubated for 72hours at 28 ±2°c. Observations were made on the growth of fungal mycelium as influenced by the plant extracts. The three extracts of *C. procera. viz,* methonal, ethyl acetate and water have exhibited antifungal activity against *Fusarium* and *T. vesiculosum.*

Introduction

Calotropis procera (R. Br.) is a semi woody weed plant belongs to Asclepidaceae, family commonly known as arka, madar, mudar etc., Procera is a spreading shrub or small tree, contains resin, cardenolids, steroid glucosides, useharin, calotoxin, and calactin. Leaves and stalks contains a bio active chemical constituents of sterols, resins, cardenolides, calotropin, calotropagenin etc., In flower the chemical constituents are the high amount of ash and proteins with varying quantities of alkaloids, and anthocyanins. Root bark and root contain bitter yellow resin but no alkaloid. The whole plant contains flavone glycosides and cardiac glycoside. The various parts of the giant milkweed tissues posses numerous medicinal properties, especially the root bark due to the presence of resin, cardenolids, steroid glucosides etc. A new cardenolide, proceragenin, has been isolated from *C. procera* showed antibacterial activity against both gram positive and gram negative bacteria (Akhtar 2006). Aqueous extract of leaf, flower and roots of *C. procera* proved most effective in the control of *Henosepilachna elaterii* by expressing strong repellent activity and thus deterred the insects from feeding (Umsalama, 2006). To evaluate the antifungal and antimicrobial activity analysis of *C. procera,* HPLC analysis of free fatty acid methyl ester and antifungal activity against *Fusurium, Trochosporium vesiculatum* using the samples of methanol extract, water extract and ethyl acetate extract was carried out.

Materials and Methods

Different parts of *C. procera* like flowers, leaves and stem were collected for extraction. Fresh weight of each plant tissues was recorded

Table 1. Effect of antifungal property of different extracts of *C. procera* tissues

C. procera (250ppm)	Inhibition of Growth (%)	
	Fusarium sp.	*T. vesiculosum*
Methanol	03.26	50.00
Ethyl acetate	54.34	18.47
Water	48.91	56.52
Control	No inhibition	No inhibition

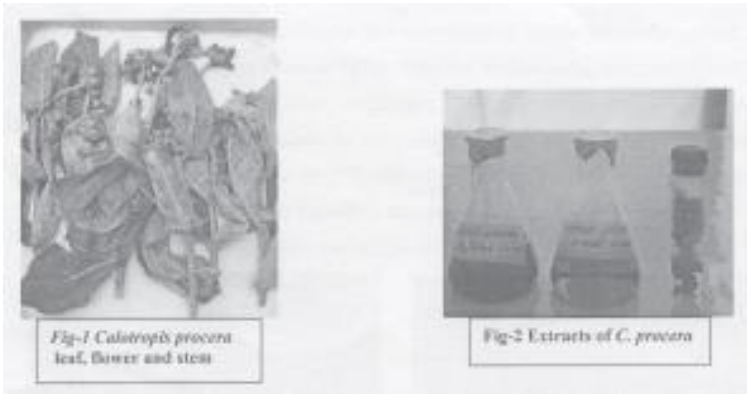

Fig-1 Calotropis procera leaf, flower and stem

Fig-2 Extracts of *C. procera*

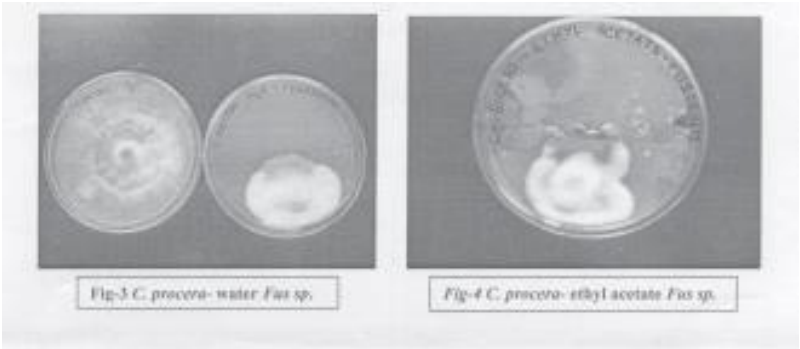

Fig-3 *C. procera*- water *Fus sp.*

Fig-4 *C. procera*- ethyl acetate *Fus sp.*

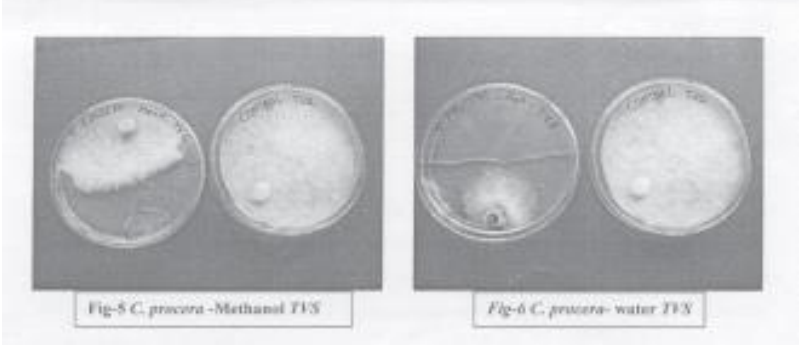

Fig-5 *C. procera* -Methanol *TVS*

Fig-6 *C. procera*- water *TVS*

and cut in to smaller pieces in order to facilitate the efficient freeze drying. Following the dry weight determination the dried parts of leaf, flower and stem were milled and stored in 20°C until extraction.

Extraction procedure

Separate extractions were conducted for each plant parts. All the plant parts are extracted with water, MeOH, Ethyl acetate; solvents were removed under vacuum and stored at -20°C till the bioassay and estimated some of the primary and secondary groups.

HPLC

The method of Schuster (1985) was followed to analyse the free fatty acid methyl esters of C. *procera* (whole plant) sample in HPLC.

Fungal Isolates and bioassay:

Isolates of fungal pathogens *viz. Fusarium* and *T. vesiculatum* were made from freshly collected disease samples. The cultures were maintained on potato dextrose agar until further study. Aliquotes of potato dextrose agar medium was poured into sterile petridishes. The petridishes were marked into two equal halves. Dishes of 5mm dia , punch from the margin of an actively growing colony of each fungal pathogens were placed in one half of the petridish on agar medium.20ul of each of the plant 250ppm extract was applied in the centre of the petridish dia. DimethyL-sulfoxide(DMSO) was used as control. The setups were incubated for 72 hours at 28 ±2°C. Observations were made on the growth of fungal mycelium as influenced by the plant exracts. Based on the growth of fungi in response to plant extract the rate of inhibition was measured in millimeter.

Results and Discussion

The results of antimicrobial activity of whole plant of *C. procera* are summarized in Table 1. (except roots, stem, leaf and flower)

All the three extracts of *C. procera,* methanol, ethyl acetate and water have exhibited antifungal activity against *Fusarium* and *T. vesiculosum.* Among them water and ethyl acetate extracts exhibited high anti fungal activity against *Fusarium* at 250ppm. Whereas, in the case of *T. vesiculosum* water and methonal extracts of whole plant exhibited toxic effect. In both the cases water extract was effective as compared to methanol and ethyl acetate against *Fusarium* and *T. vesiculosum* respectively. The present investigation suggests that the whole plant of *C. procera* possesses potent antifungal activity that could inhibit the growth of fungi. It is interested to note that fatty acids of varying chain lengths are known for the antimicrobial action primarily against gram positive bacteria and yeast (Freese 1973). Toxicity studies using fatty acids have been well documented by the earlier researchers during bacterial and yeast alcoholic fermentations of different extracts. In the present study, we report the wider ability of linoleic, linolenic and palmitic acids to inhibit the fungal isolates of *Fusarium sp.* and *T. vesiculosum.* Davidson (1999) identified that oleic acid (C 18:1) had been found to be fungi-static against the wide spectrum of saprophytic moulds and yeast. Sehgal *et al.* (2005) found that antifungal property of *C. procera* is due to the presence of enzymes and stable cysteine proteases present in the latex. Akhtar (2006) isolated a new cardenolide, proceragenin from *C. procera,* which exhibited antibacterial activity against both Gram positive and Gram negative bacteria. Larhsini *et al.* (2001) found that the n-butanol extract of *C. procera* flowers and the aqueous extract *of Eugenia caryophyllata*

proved to be the most effective against the bacteria. Sharma and Trivedi (2002) reported that *C. procera* showed maximum antifungal activity against *Fusarium oxysporumf. sp. cumini.* Tahir *et al.* (2002) observed more antimicrobial activity with the leaf extract, than root bark due to the presence of saponins, glycosides, and simple sugars in the leaves, while the root bark was found to contain tannins in addition to these groups. It can hence been concluded that the three fatty acids methyl esters oleic acid, linolenic acids, Linoleic acid stearic acid and palmitic acids (Table 2) can be used in certain proportion routinely to prevent some fungal infections.

The three extracts of C. *procera,viz.*, methonal, ethyl acetate and water have exhibited antifungal activity against *Fusarium* and *T. vesiculosum.* Among the three, water and ethyl acetate extracts exhibited high antifungal activity against *Fusarium* at 250ppm. Whereas, in the case of *T. vesiculosum* water and methonal extracts of whole plant exhibited toxic effect.

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