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EFFICACY OF *SOLANUM XANTHOCARPUM* SCHRAD. & WENDL. FRUIT EXTRACT ON PHYTOPATHOGENIC FUNGI

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ABSTRACT

The objectives of this work was to study the screened of antifungal activity and minimum inhibitory concentration on phytopathogenic fungi using petroleum ether and benzene fruits extracts of *Solanum xanthocarpum* (Solanaceae). Both petroleum ether and benzene extracts of *Solanum xanthocarpum* fruits exhibited an appreciable inhibition on all the studied fungi. However petroleum ether fruit extract of *Solanum xanthocarpum* inhibited *Colletotrichum gleosporoides*, *Curvularia lunata* and *Alternaria alternata* at 900µg/ml except *Macrophomina phaeseolus* and *Sclerotium rolfsii.* Even at 100µg/ml concentration it shows 72.5% of inhibition in *Colletotrichum gleosporoides*. The MIC for petroleum ether extract was >90µg/ml, whereas for *Alternaria alternata* it was >30 µg/ml.

Key words: *Solanum xanthocarpum*, Fruit extract, Antifungal activity, Minimum Inhibitory Concentration.

INTRODUCTION

Plant based antimicrobials remain a vast untapped source for medicine with enormous therapeutic potentials. They are effective in the treatment of infectious diseases, while simultaneously mitigating many side effects that are often associated with synthetic antimicrobials. Herbal products are promisingly important source for therapeutics and may be a viable solution for disease control (Murray, 1995). *Solanum xanthocarpum* Schrad & Wendl., a member of solanaceae family is commonly called as Kandan-Kattiri and yellow night shade. It plays an important place among medicinal herbs, especially in India since ancient times. The plant found well versed in India (Gunaselvi *et al*., 2010). Ayurveda described them as aperients, pungent, bitter, digestive, alternative and astringent. The herb is anthelmintic, expectorant and stomachic. The plant is useful in fever, cough, asthma, constiveness and pain in chest (Chopra *et al*., 1992). The stem, flower and fruits are used for vesicular and watery eruption. Juices of the berries are useful in sore throat has reported to possess beneficial activity in asthma and chronic bronchitis and also cardiac stimulatory activity (Govindan *et al*., 2009; Gupta *et al*., 1967).

Fruits of *Solanum xanthocarpum were* investigated against *Aspergillus niger* and *Trichoderma viride* (Singh *et al*., 2007). Aqueous extract of fruit of *Solanum xanthocarpum* was investigated for hypoglycemic activity in rats and mice. Its fatty oil also possesses significant antifungal activity (Kar *et al*., 2006).

Major chemical constituents present in plant: Solasodine (Verbist *et al*., 1947), Steroidal alkaloids (Kusano *et al*., 1973), β-Solamargine, β-Solamargine, Solasonine, Sterols Viz., Cycloartenol, Norcarpesterol, Cholesterol (Kusano *et al*., 1975), Solanocarpine, Carpesterol, Atropine, Diosgenin and β-Sitosterol (Heble *et al*., 1968), 3-Hyhydroxy-3 Methyl Glutaryl COA Reductase, Solanine, Glycosides, Phenolics, Flavonoids, Steroid and Saponins (Amir and Kumar, 2004). Since, the antifungal efficacy of *Solanum xanthocarpum* has not been studied extensively. So, the present attempt was taken to study the screening of antifungal activity and

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minimum inhibitory concentration on phytopathogenic fungi using *Solanum xanthocarpum* (Solanaceae) fruit extracts.

MATERIAL AND METHODS

Plant Material

Fresh fruits of *Solanum xanthocarpum* (Solanaceae) were collected from waste land around Chennai, Tamil Nadu, India. The plant material was identified in the Department of Plant Biology and Plant Biotechnology and a voucher specimen (SX12) were deposited and are maintained in the Department's Herbarium. Freshly collected fruits were washed thoroughly and shade dried in open air and grounded into powder.

Extraction of Plant Material

The powdered plant (50g) was suspended with twice the volume of petroleum ether and benzene separately in a Soxhlet apparatus and the crude extract was concentrated. The contents were stored at 4°C in air tight containers for future use.

Microorganisms Used

Colletotrichum gleosporioides, Alternaria alternata, Curcularia lunata, Macrophomina phaseolus, Sclerotonia rolfsii were obtained as "gratis" from Centre for Advanced Studies in Botany, University of Madras, Chennai and maintained on PDA (Potato Dextrose Agar) slants at 28± 2ºC.

Antifungal Activity

Antifungal activity was screened using poison food technique (Dhingra and Sinclair, 1985), and minimum inhibitory concentration (MIC) was determined by broth dilution method. Briefly, different concentrations of petroleum ether and benzene extracts such as 100µg, 300µg, 500µg, 700µg, 900µg/ml respectively, amended into potato dextrose agar medium and the fungi were inoculated and maintained at room temperature and the radial growth was determined after 5days.

MIC was carried out in potato dextrose broth with petroleum ether extract with a concentration ranging from 10 to 90µg/ml. The growth of the fungi was assessed visually after 48 hrs of incubation.

Effect of Plant Extract on Dry Weight

Petroleum ether extract amended with different concentrations (10-100µg/ml). Disc of 5mm (dia) of *Alternaria alternata* and *Colletotrichum gleosporoides* was inoculated into each flask. After a week the mycelial mat was separated by filtering using a pre-weighted whatman No: 1 filter paper. The mycelial mat was dried in hot air oven. The dry weight was calculated by taking the difference in final weight and initial weight of the filter paper.

Cellulase Assay- (Fpa) Filter Paper Assay

Enzyme assay was determined according to modified method (Mandels, 1974). Sugar cane bagasse was mixed with petroleum ether extract (100µg and 300µg). Two discs of 5mm diameter of the fungi (*Colletotrichum gleosporioides*) were inoculated into each flask. After 10 days of growth of the mycelium, the bagasse was extracted with 20ml of citrate buffer to obtain the extracellular enzyme. 6 x1cm long whatman No: 1 filter paper was placed in the test tube along with 1 ml of buffer and 2ml of enzyme and the reaction mixture was incubated in a water bath maintained at 50˚C for 1 hour. 1 ml of the solution was taken and 2ml of anthrone reagent was added and the blue coloured complex was read in a colorimeter at 600 nm. The absorbance was plotted on to the graph. Enzyme activity was expressed as U/mg protein.

Thin Layer Chromatography

An aliquot of crude petroleum ether extract was plotted on a pre-coated silica gel TLC $60F_{254}$. The spot was allowed to dry and the plate was developed in a solvent system containing 2:8 ratio of diethylether and benzene. The chromatogram was developed and observed under long UV 365 nm and the Rf values of the spots were recorded. The plates were also sprayed with 1:10 ratio formaldehyde and conc. Sulphuric acid to detect the various compounds present in it.

TLC Bioautography

This inhibition activity was determined by the procedure used (Lago *et al*., 2004). An aliquot of petroleum ether extract was spotted on a precoated silica gel TLC plate and developed using petroleum ether. Spores suspension of *C. gleosporoides* was prepared in 2% glucose solution and it was sprayed on to the TLC plate and incubated in moist chamber. After 48 hours, appearance of inhibition zone around the individual spot was observed.

RESULTS AND DISCISSION

The yield of petroleum ether and benzene crude extracts was 2.58g and 2.73g respectively**.** It was evident from the above study that *Solanum xanthocarpum* fruit extracts effectively inhibited the plant pathogen. Petroleum ether extract at 900µg/ml showed lethality to all the fungi except *Macrophomina phaeseolus and sclerotium rolfsi. Colletotrichum gleosporoides* was inhibited effectively even at 100µg/ml, where the inhibition was 72.5%. whereas in benzene extract *Macrophomina phaeseolus* was alone inhibited to 100%

at 900µg/ml while all other fungi was inhibited to an extent of 50%. Petroleum ether extract demonstrated effective antifungal activity when compared to benzene extract. The antifungal effect of petroleum ether extract and benzene is tabulated (Table No. 1 & 2). The petroleum ether possessed certain metabolite which was highly toxic to anopheles species at 0.9mg/ml. This indicates that such metabolite probably is present in petroleum ether extract (Mohan *et al*., 2007). Hence it exhibited higher inhibition activity.

It has been reported that benzene extract of *S. xanthocarpum* showed no inhibition on bacteria, while acetone extract had antibacterial activity (Palavesam *et al*., 2006). In the present study, benzene extract induced inhibition on the test pathogens. The antifungal properties of methanolic extract *of S. xanthocarpum* on *Aspergillus niger* and *Trichoderma vivide.* Where it had inhibited the radial growth effectively.

The methanolic extract of *S. xanthocarpum* exhibited inhibition *on Aspergillus fumigatus, A. flavus* and *A.niger* (Singh *et al*., 2007). In the present study the petroleum ether extract induced appreciable inhibition at 0.1mg/ml on the pathogen studied. A similar observation was reported that methanolic extract exhibited inhibition on fungi at 0.125mg/ml (Dabur *et al*., 2004).

The minimum inhibitory concentration of petroleum ether extract concentrated below 100µg (10µg, 30µg, 50µg, 70µg, 90µg/ml) showed significant inhibition on the plant pathogen. Over all it was observed that the MIC ranged between 0.30-0.90mg/ml for all plant pathogen. Whereas, for *Alternaria alternata* the MIC was >30µg/ml. The methanolic extract had a MIC of 1.25 - 2.5mg/ml on *Aspergillus fumigates , A. flavus* and *A. niger* (Dabur *et al*., 2004). However the petroleum ether extract tested against the pathogen in the present study revealed a lesser MIC ranging from 0.30 - 0.90 mg/ml. Hence it is postulated that the toxic principle or phytochemicals present in the petroleum ether is solely responsible for the inhibition of the test fungi.

The petroleum ether extract showed high inhibition on *Colletotrichum gleosporoides* and *Alternaria alternata* when compared to that of benzene extract. Hence further studies carried out on *Colletotrichum gleosporoides* and *Alternaria alternata* with petroleum ether extract.

The effect of petroleum ether extract on the dry weight of *Alternaria alternata* demonstrated lethal action at 0.9mg/ml, where maximum inhibition obtained was (94.8%) than other concentration (0.1mg, 0.3mg, 0.5mg and 0.7mg/ml). *Colletotrichum gleosporioides* was significantly inhibited by petroleum ether extract, where the inhibition was 93.3% at 0.9mg/ml . However petroleum ether also exhibited inhibition at lower concentration of 0.1mg/ml (Fig. 1).

C. gleosporoides is seed borne pathogen causing anthracnose disease resulting in great yield loss in plants. Pesticides and chemical used to control pathogen causes environmental pollution. Hence it is suggested that such plant based biopesticide can be used as an alternative control measure. *Colletotrichum gleosporioides* showed maximum inhibition on dry weight when compared with *Alternaria alternata*. Hence further studies carried out on *Colletotrichum gleosporioides.*

The petroleum ether extract at concentration 100µg and 300µg showed a reduction in the cellulase. The filter paper away revealed that the cellulase activity in the control was 32u/mg protein. And 100mg/ml, 300mg/ml had 12U/mg and 25u/mg protein. Hence petroleum ether extract inhibited cellulase activity (Fig. 2).

The TLC plate was sprayed with 1:10 ratio formaldehyde and concentrated sulphuric acid to observe different colour spots. Four different compound were observed on TLC plate with an Rf value of compound A – 0.25, Compound B – 0.32, compound C – 0.45 and compound D – 0.60. When the TLC plate were observed under the UV 365nm, one major blue fluorescent spot with Rf value of 0.60 was observed. It has been reported that the presence of 7 spot in ethanolic extract (Li *et al*., 2005). However the present study revealed the one fluorescent spot under UV and 4 spot in the spraying reagent (Fig. 3 A & B).

Table 1. Effect of petroleum ether fruit extract on the radial growth of fungi

S.No.	Organism	Radical growth in mm					
		Control	**100 µg**	**300 µg**	**500 µg**	**700 µg**	**900 µg**
1	*Curvularia lunata*	6.5	4.5(31%)	4.2(35%)	4(38%)	3(54%)	0(100%)
2	*C. gleosporoides*	14.5	4(73%)	4(73%)	3.5(76%)	3.2(78%)	0(100%)
3	*Alternaria alternata*	13.5	4.2(69%)	3.5(75%)	3(78%)	3(78%)	0(100%)
4	*Macrophomina phaseolus*	39.2	22(44%)	16(59%)	14.2(61%)	8(79%)	1.5(81%)
5	*Sclerotium rolfsii*	32	23(28%)	17(47%)	15(53%)	12(63%)	8(75%)

All values mean of radial growth. Values in parenthesis - inhibition percentage

Table 2. Effect of benzene fruit extract on the radial growth of fungi

S.No.	Organism	Radical growth in mm					
		Control	100 µg	300 µg	500 µg	700 µg	900 µg
1	*Curvularia lunata*	63	27(73%)	22(77%)	21(79%)	14(85%)	11(88%)
2	*C. gleosporoides*	80	26(73%)	28(72%)	24(76%)	19(81%)	15(85%)
3	*Alternaria alternata*	14	3.8(62%)	3.7(62%)	3(70%)	2.4(75%)	2(77%)
4	*Macrophomina phaseolus*	18	3.3(66%)	3(68%)	2.8(72%)	2.4(76%)	0(100%)
5	*Sclerotium rolfsii*	60	34(43%)	32(47%)	27.6(54%)	17.3(71%)	10.2(83%)

All values mean of radial growth. Values in parenthesis - inhibition percentage

Fig 1. Dry Weight of *Alternaria alternate* and *Colletotrichum gleosporoides*

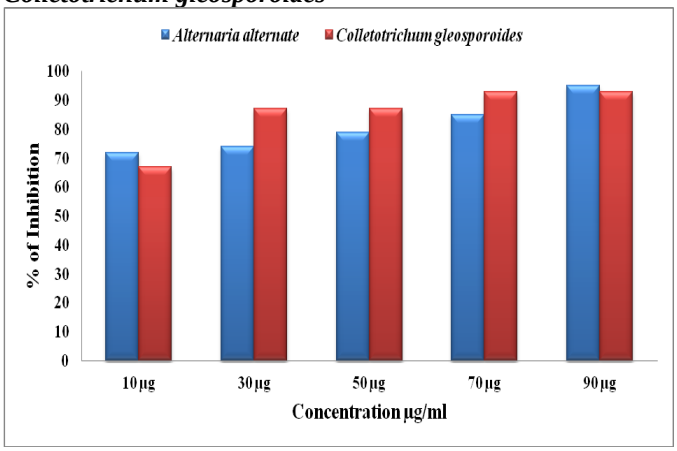


Fig 2 .Cellulase Activity of Petroleum Ether Extracts

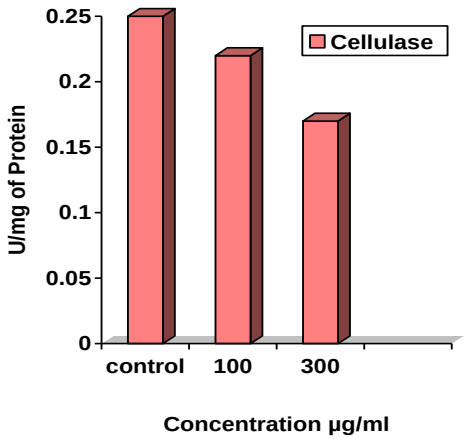
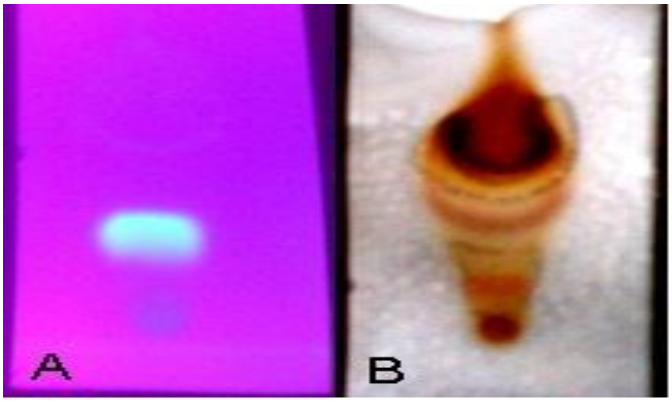


Fig 3. Thin Layer Chromatography of Petroleum Ether Extract



TLC Of Petroleum Ether Extract

A) One major blue fluorescent spot observed under 365 nm

B) TLC plate sprayed with detecting reagent, four different compound were observed on TLC plate with an Rf value of compound A-0.25, Compound B-0.32, compound C-0.45 and compound D-0.60.

The developed TLC plate with petroleum ether extract exhibit four compound with the RF value of 0.25 ,0.32, 0.45 and 0.60 . The TLC bioautography for the petroleum ether extract showed the inhibition of C*olllectotrichum gleosporiodes* by the 4th compound which exhibited characteristic blue fluorescence with RF value of 0.60. Studies showed that dichloromethane extract showed no Inhibition on *Curvularia lunata* and *Alternaria alternata* using direct bioautography (Sanjay Guleria *et al.*, 2006). While in the present work *Colletotrichum gleosporoides* showed inhibition on petroleum ether extract.

CONCLUSION

Several plant disease caused by fungi which affect the plant crop, result in loss and decrease the quantity and safety of agricultural product are generally controlled by chemical that are subjected to strong restriction and regulation. Plant antimicrobials are effective and ecofriendly, hence it is concluded that petroleum ether fruit extract of *Solanum xanthocarpum* can be effectively utilized as biopesticide against plant pathogens to decrease the yield loss in agricultural product.

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