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Comparative Analysis of the Antifungal Activity of Different Solvent Extracts of *Uvaria narum* (Dunal) Wall. Against *Fusarium moniliforme* and *Corynespora cassiicola*

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ABSTRACT

The antifungal potential of one of the lesser known members of Annonaceae, Uvaria narum (Dunal) Wall. prominently found in Western Ghats of Kerala and Mangalore, were tested against two important plant fungal pathogens, Fusarium moniliforme that causes Leaf Rot in Coconut, and Corynespora cassiicola, the causagent of Leaf Fall in Rubber. The leaves of Uvaria narum were subjected to sequential soxhelet extraction in four solvents, ie, Petroleum Ether (PE), Chloroform (Chl), Acetone (Ac) and Methanol (Me). The extracts thus obtained were subjected to antifungal tests by Poison Food Technique. The inhibition percentage was noted for every extract. A very good antifungal potential was exhibited by the Petroleum Ether and Chloroform extracts of U.narum against both the tested fungus while acetone and methanol extracts showed no inhibition at all. The hot soxhelet extractions of PE showed better inhibition to the growth of both the fungi than cold extraction in PE thereby proving that the compound with antifungal potential was better extracted by soxhelet method than the cold method of extraction. It could also prove that the bioactive compound was thermally stable.

Key words: *Sequential Soxhelet Extraction, Inhibition, Thermally stable, Poison Food Technique, Causagent*

INTRODUCTION

The Annonaceae family has been recognized as a potential source of insecticidal substances. There are a large number of chemical substances in different species. The group of chemical substances that have grabbed the maximum attention is the class of substances called as acetogenins. Also called ACGs, these compounds are a series of naturally occurring secondary metabolite products (C-35/C-37) derived from long chain fatty acid and combined with a 2-propanol unit.[1] A great deal of work in several members of Annonaceae has been done for which a lot of literature is available. But there are still members that are unexplored and unexploited and whose bioactivities or phytochemical properties remain unknown.

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Uvaria narum is one such plant who's phytochemistry, though worked out to a great extent, remains ambiguous in terms of bioactivity. The following work was undertaken to evaluate the antifungal potentialities of various extracts of *Uvaria narum* obtained by sequential method of soxhelet extraction. The fungi used were '*Fusarium moniliforme'* that causes Leaf Rot in Coconut, and *Corynespora cassiicola*, the causagent of Leaf Fall in Rubber.

Fusarium moniliforme, belonging to order Hypocreales of Phylum Ascomycota is the anomorph of *Gibberella moniliforme* (fujikuroi) and is a soil residing fungus. It produces toxins called Fumonisins.[2] While *Fusarium* species are mainly associated with the root and stem rots , in coconut palm they have been found to be important causagents of Leaf Rot Diseases (LRD). Though several agents were responsible for causing leaf rot in coconut palm it was established by Srinivasan *et al*, that *Fusarium* spp. were instrumental in developing this LRD disease during high temperature, less humidity conditions prevalent during the months of January-March[3].

Corynespora cassiicola, (Berck. & M.A.Curtis) C.T.Wei, which is a common causagent for Corynespora leaf fall, (CLF)[4] is one of the most economically significant fungal diseases on cultivated rubber trees *Hevea brasiliensis* in Asia and Africa. It is a member of mitosporic Ascomycota, the Deuteromycetes or imperfect or asexual fungi that is to say, like *Fusarium*, it lacks a sexual or a teleomorphic state. Both these fungi are of great economic importance to a state like Kerala, which cultivates rubber as its main cash crop and coconut as its main plantation crop[4].

Uvaria narum is a woody climbing shrub, with solitary flowers, bearing scarlet fruitlets. Also known as Kooril and Narumpanal in Malayalam dialect, this plant though proclaimed to be abundant in the plains and deciduous forests of Western Ghats in Kerala and Karnataka at lower altitudes, is now being removed from its prominent areas due to vast scale constructions. The number of this species has come down to such an extent that this member has got confined only in the protected areas of campuses, or temples or sacred groves. Though this plant has not been subjected to a thorough screening for insecticidal properties, some reports have been available endorsing its significance as an effective antifungal by the virtue of prescence of abundant acetogenins, isolated primarily by Hisham and his team,[5]. The acetogenins found in *Uvaria narum* include uvariamicins I,II and III,[6] squamocin, squamocine -28-one and Panalicin,[7] isodesacetyluvaricin, narumicin I and II, [8] in addition to known compounds, glutinone, glutinol, taraxerol, and , β-sitosterol and benzyl benzoate.

In a study conducted by Padyana Subrahmanya on the Antibacterial and Antioxidant properties of *Uvaria narum* (Dunal) Wall. the root extract in various solvents showed inhibitory properties against both Gram positive and Gram negative bacteria, *Staphylococcus aureus, Escherichia coli, Bacillus* species and *Lactobacillus fermentum*, thereby justifying its use as a cure against skin ailments by certain tribes.[9] It is used for gastro intestinal ailments by traditional medicinal practitionars from Kalrayan Hills of Villupuram district in Tamil Nadu.[10] Roots and leaves used in intermittent fevers, biliousness, jaundice, also in rheumatic affections; and is used in skin diseases. A decoction of the root bark is given to women to control fits at the time of delivery.[11]

Objectives of the chapter:

- To conduct an in depth study of the antifungal activity of various extracts of *Uvaria narum* obtained by sequential soxhelet extraction in various solvents of different polarities.
- To confirm whether the antifungal compound is thermolabile by conducting experiments in both hot (soxhelet) and cold extraction, in at least one solvent

MATERIALS AND METHODS

The *Uvaria narum* leaves were collected in the month of November, 2015 from Kottayam

district of Kerala. The material was identified as the desired plant from S.B College, Changanacherry, Kerala. Some specimen were dried and prepared as herbarium. The soxhelet extraction of the *Uvaria narum* air dried and powdered leaves were done in four solvents *viz*, Petroleum ether, chloroform, acetone and methanol, sequentially as well as individually. To confirm whether the soxhelet extraction gave better results than the cold ones, the growth of the *Fusarium moniliformis* and Corynesporium fungi were tested both on cold pet ether extract and hot Pet ether extracts. The fungus *Fusarium* was kindly obtained from CPCRI, Kayamkulam, Kerala, and *Corynespora cassiicola* was obtained from Rubber Research institute, Puthuppally. The medium used for culturing and subsequent subculturing of the fungi was Potato Dextrose Agar (PDA). 24 gm of readymade Potato Dextrose Broth (PDB) (Merck.), was dissolved in one litre of distilled water and 15gms of agar was dissolved to prepare the required PDA medium.

The extracts were dissolved in various solvents and acetone was found to be the best solvent to dissolve all forms of extracts[12]. It was preffered at 2% of the total media strength. The inhibition studies were conducted by using poison food technique as suggested by Nene and Thapliyal[13]. A specific amount of extract (max.5mg/ml) was added to the PDA and after swirling nicely was poured into the petriplates. Once it solidified, discs of 5mm diameter from the periphery of 5 to 7 day old fungal plates were cut and kept on the solidified PDA at the centre and plate covered. Control plates were also set with the respective solvents as +ve and distilled water as the negative control. The growth of hyphae was measured by scale in cm. at right angles till the last but one day when the hyphae fully covered the plate and compared to the standard. % growth was taken with the formula:

I %= C-T/C x 100

Where C= radius of hyphae in control
T= radius of hyphae in extract
I= Inhibition %

If the extract showed an inhibition more than 50%, it was said to be effective in nature.

The statistical analysis of testing the significance was done by MSExcel. All the experiments were repeated six times and significance tested at $p<0.05$ level.

RESULTS

The yield of the Petroleum Ether extract by Cold Extraction method was 0. 470gms/35gms and it was 0 .750gms/35gms.by soxhelet method of extraction. There was a significant difference in the percentage inhibition of activity between the two extracts at 5% significance level, with the soxhelet extract of PE showing a better activity than cold extract of PE against both the fungi tested. The controls both positive and negative showed the minimum inhibition, and the maximum growth. (Table1.) Also among the sequential extracts, the sequentially extracted PE extract and the chloroform extract derived thereafter inhibited the fungus growth by 65% and about 49% in case of Fusarium and 70% and 45% in case of Corynespora. The acetone and the methanol extracts derived sequentially did not exhibit any inhibition to the fungi tested. (Table 2.)

Table 1: Comparison of inhibition activities of hot and cold PE extracts of *Uvaria narum* leaves against *Fusarium* and *Corynespora*

	Mean±SE	STD.DEV	df	t-stat
Fu(Hot) PE	65.833±0.86	2.12	9	10.65*
Fu (Cold)	49.866±1.22	2.99		
Co(hot)	70.91±1.87	5.2	10	8.8*
Co(cold)	45.94±5.2	5.2		
Fu(water)	-			
Fu(acetone)	-			
Co(water)	-			
Co(acetone)	-			

Table 2: Comparison of inhibition activities of Sequential Soxhelet extracts of PE, Chl, Ac and Me of *Uvaria narum* leaves against *Fusarium* and *Corynespora*

	Fusarium				***Corynosporium***			
	(PE)	(Chl)	(Ac)	Me	(PE)	(Chl)	(Ac)	Me
Mean±SE	63.84±0.11*	47.97±1.01*	0	0	61.84±0.11**	53.68±0.49**	0	0
Std.Dev	0.744	1.01	0	0	0.7	2.98	0	0
*t-stat	5.155*							
**t-stat					6.52**			

- Both acetone and water showed zero inhibition and a maximum growth.
- '*' Significance at $p<0.05$

DISCUSSION

The extracts prepared in Petroleum Ether by soxhelet method of extraction gave a better inhibition of the tested fungus than the extracts prepared by cold method of extraction. This could be because the active compound got extracted more in the soxhelet form of extraction (2.4% of yield), than in the cold extract (1.28% yield). Also, it proved that the active compound was not thermolabile in nature. This rightly goes with the statement that "extraction of compounds from the plants is an empirical exercise where different solvents are utilized under a variety of conditions such as time and temperature of extraction". [14] Thus it was concluded that the Soxhelet method of extraction was the apt method to extract maximum amount of bioactive compounds from this plant for its antifungal studies. It was found that the maximum activity was shown by the PE and Chl extracts (sequentially obtained). Also, it was noted that there was absolutely no inhibition activity in the acetone and methanol extracts derived sequentially. This could only mean that the bioactive compound was completely extracted by the petroleum ether and chloroform solvents and no antifungal activity containing compounds were present in the remaining extracts.

CONCLUSION

Among all our agricultural produces, around 10 to 20% of staple food and cash crops are currently being destroyed by plant pathogens[15]. It's imperative that we try to find out a long term solution to all these problems. Leaf rot of Coconut and Leaf fall of Rubber are two persistant fungal problems of cash crops Kerala has to face. Annonaceae family is a family of tropics, with several unexploited species still remaining to be explored. *Uvaria narum* happens to be one such plant that is still underexploited. Its antifungal potentialities against the above mentioned fungi were tested using extracts obtained from four different solvents of different polarities. While the petroleum ether and chloroform extracts gave satisfactory results, no inhibitions were shown by the acetone and methanol extracts. The antifungal component present in the PE and chloroform extracts was not thermolabile in nature too. Bioactivity guided fractionation studies are going on to find out whether the antifungal compound is acting alone or its synergistic action of a group of compounds. Also efforts are going on to isolate and purify this compound and characterise it to find out its true chemical nature.

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