ANTIFUNGAL ACTIVITY OF *Plectranthus zeylanicus*

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Abstract

In a continuation of study towards the discovery of biologically active compounds from Sri Lankan medicinal plants, we have chemically investigated some of the selected plants for their antifungal activity. Preliminary investigations showed that methanol extract of the plant Plectranthuszeylanicus showed significant antifungal activity. The bioactive agent of the above mentioned plant in the most potent fractions with antifungal properties were identified using column chromatography, eluted using gradient elution technique using three different solvents as a mixture of two in different ratios as a combination of hexane: ethyl acetate, ethyl acetate: methanol in increasing polarity, followed by antifungal assay against Aspergillus species for each fractions. Analysis of Antifungal activity profile of the plant showed that the highest inhibition activity in (75% Hexane: 25% Ethyl acetate) and (80% Ethyl acetate: 20% methanol) fractions and the respected value of the highest inhibition diameter was found to be 12.83mm for both fractions. Further isolation of these fractions are under study and introduction of chromatographic techniques into this work will possibly pave the way for an effective discovery of an antifungal agent. The obtained results provide a support for the use of this plant in traditional medicine and it would be a potential antiseptic source for the prevention and treatment of fungal infections.

Keywords:*Plectranthuszeylanicus*,column chromatography,gradient elution technique, antifungal assay, *Aspergillus*.

Introduction

Human and plant fungal infections pose serious medical issues. Up to now, more than a hundred thousand fungal species are considered as natural contaminants. There is a general consensus among researchers, clinicians and pharmaceutical companies that new, potent, effective and safe antifungal drugs are needed. Historically, most of the substances have been part of natural products. Therefore, it is quite logical that any recent search for new prototype antifungal products should also include a variety of plant extracts. In designing a search for novel prototype antifungal agent, it seems reasonable to assume that if new agents are to be found that have different structures and different activities from those in current use, higher and lower plants are a logical choice. It is chiefly because of their seemingly infinite variety of novel molecules, which are often referred to as secondary metabolite (Clark and Hufford, 2010).

Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of their having normally matchless chemical diversity. Natural products are also associated with low levels of toxicity, and in many cases have a fairly broad spectrum of activity (Silver and Bostian, 1990).
In recent years, a large number of synthetic antifungal agents has been banned in western world because of their undesirable attributes such as high and acute toxicity, long degradation period, accumulation in food chain and an extension of their power to destroy both useful and harmful organisms.

Considering these undesirable attributes of synthetic fungicides, there is an urgent need to develop alternative treatments that are less hazardous to human, animals and plants and

that impact less on the environment. Within this context, plant produced compounds are of interest as a potential source of safer and more effective substitutes for synthetically produced antifungal agents. Extracts isolated from several plants have been reported to have antifungal activity.

Therefore the purpose of this study was to evaluate the *in vitro* antifungal activity of the extracts from the plant *Plectranthuszeylanicus*against pathogenic *Aspergillus* Species.

Materials andMethods

Collection and identification of plant
Fresh leaves of the plant specimen were collected from Mawanella which is in Kegalle district from Sabragamuva province of Sri Lanka in March, 2016.

Preparation of plant for extraction
The leaves of *Plectranthuszeylanicus* were first washed under running tap water followed by sterile distilled water. Then they were shade dried under room temperature until all the water molecules evaporated and ready for grinding. The leaves were ground using mechanical grinder into fine powder and was transferred into airtight container.

Extraction of the leaves
The powdered leaves (122g) were macerated in 1000ml methanol for 24 hours and then mixed well for 12 hours using mechanical stirrer. Then, the solution mixture was filtered and poured into round bottomed flask and put in the rotary evaporator under reduced pressure at (30-40) °C until the solvent got evaporated. A semi-solid, plant extract was obtained, which was placed in a beaker and weighed (RashaSaad*et al* 2015).

Fractionation using column chromatography
A slurry was prepared with crude extract (9g) dissolved in minimum quantity of dichloromethane, mixed with silica gel 60 (18g) and dried by using rotary evaporator under reduced pressure at (30-40) °C. Obtained residue was finely powdered by using mortar and pestle and was ran through the column packed with Silica gel G (60-120) as a stationary phase(Bristow*et al* 1977). The column was eluted with solvents such as hexane, ethyl acetate, methanol in different ratios in a combination of hexane: ethyl acetate and ethyl acetate: methanol in increasing order of polarity by 5 ml (and in some fractions by 25 ml due to high polarity and very slow elution) making sure that the total volume of solvent mixture remained 100 ml.

Assay of antifungal activity against *Aspergillus* species
Disk diffusion method was slightly modified in order to use for filamentous fungi like *Aspergillus.* In this method, a liquid culture of *Aspergillus* on CDB was prepared by inoculating 7 day old fungus grown on PDA.

Sterile disk papers (Diameter 6 mm) were soaked in the test samples dissolved in methanol in order to get 200 µg of the sample per a disk(0.02g of the crude extract was dissolved in 100µl and 5µl was pipetted out in order to get 200µg of the sample). Meanwhile, CDA medium was prepared, autoclaved and cooled to about 45°C and then inoculated with the liquid culture of *Aspergillus* species (0.5 ml of liquid culture for 25 ml of PDA medium). Then the medium was poured into sterilized petri plates (20 ml per each) and left until solidify. After solidification, dried disk papers were placed on the

inoculated medium and the plates were kept in a refrigerator at 4°C.After 24 hoursthe plates were transferred into an incubator (30°C). Diameter of inhibition zones were measured along the two axes at right angles to each other. Three replicates were used for each sample and methanol was used as negative control (Senguttuvan*et al* 2014).

Results and Discussion

Antifungal activity of test samples were observed after 3 days.The methanol extract of *Plectranthuszeylanicus*showed inhibition zones in 10 fractions, 4 from the combination of Hexane: Ethyl acetate and 6 from Ethyl acetate: Methanol fraction.

Table 1. Antifungal Activity of Hexane: Ethyl acetate test samples

Sample No	Hexane : Ethyl acetate fraction		Test Result
	Hexane (%)	Ethyl acetate(%)	
E1	100	00	IN
E2	95	5	IN
E3	90	10	IN
E4	85	15	IN
E5	80	20	IN
E6	75	25	IP
E7	50	50	IP
E8	25	75	IP
E9	00	100	IP

Where, IN- Inhibition Negative, IP- Inhibition Positi

The results of the present study present an easy in vitro system that can be used for assessing the antifungal activities of medicinal plants. Freiburghans et al. (1996) reported different solvent extracts of some plants to have different pharmacological properties. Here we concentrated on the methanol extract of this plant and was able to find significant antifungal fractions which were fractionated from column chromatography.

The maximum zone of inhibition was found in two fractions: (75% Hexane: 25% Ethyl acetate) and (80% Ethyl acetate: 20% methanol)and the value of the inhibition diameter was found to be 12.83mm.

Significant inhibition zone was also found in (75% Ethyl acetate: 25% methanol) fraction and the respected value was 11.42 mm.

Similarly (50% Hexane: 50% Ethyl acetate) and (70% Ethyl acetate: 30% methanol) fraction showed inhibition diameter of 11.00 mm. And the rest of the fractions showed 10.67 mm, 10.33 mm, 9.42 mm, 9.25 mm and 6.33 mm.

The control sample (Methanol) which was used as negative control did not show any inhibition zones.

Table 2. Antifungal Activity of Ethyl acetate: Methanol test samples

Sample No	Ethyl acetate : Methanol fraction	Test Result

	Ethyl acetate (%)	Methanol (%)	
M1	95	5	IN
M2	90	10	IN
M3	85	15	IP
M4	80	20	IP
M5	75	25	IP
M6	70	30	IP
M7	65	35	IP
M8	60	40	IP
M9	55	45	IN
M10	50	50	IN
M11	45	55	IN
M12	40	60	IN
M13	35	65	IN
M14	30	70	IN
M15	25	75	IN
M16	20	80	IN
M17	15	85	IN
M18	10	90	IN
M19	05	95	IN
M20	00	100	IN

Where, IN- Inhibition Negative, IP- Inhibition Positive

Table 3. Average inhibition diameter of active fractions

Inhibition fractions of *Plectranthuszeylanicus*&Control	**Average Inhibition Diameter (mm)**
75ml Hexane + 25ml EthylAcetate	12.83
50ml Hexane + 50ml EthylAcetate	11.00
25ml Hexane + 75ml EthylAcetate	9.42
0 ml Hexane + 100ml EthylAcetate	9.25
85ml Ethyl Acetate + 15ml Methanol	6.33
80ml Ethyl Acetate + 20ml Methanol	12.83
75ml Ethyl Acetate + 25ml Methanol	11.42
70ml Ethyl Acetate + 30ml Methanol	11.00
65ml Ethyl Acetate + 35ml Methanol	10.67
60ml Ethyl Acetate + 40ml Methanol	10.33
Methanol (control)	0.00

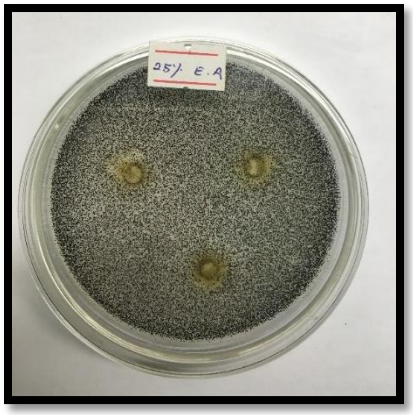

Figure 1. Test sample with 75% Hexane and 25% of Ethyl acetate

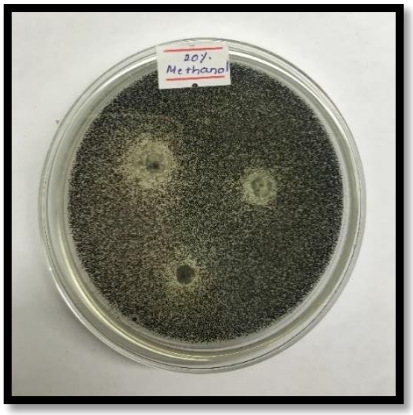

Figure 2. Test sample with 80% of Ethyl acetate and 20% Methanol

The picture of the two fractions which showed the highest inhibition diameter:

Conclusion

The present study revealed that the extract of *Plectranthuszeylanicus*possess significant antifungal activity and it leads to discover novel antifungal drugs. Analysis of antifungal activity profile of the plant against *Aspergillus* showed that highest inhibition activity is present in (75% Hexane: 25% Ethyl acetate) and (80% Ethyl acetate: 20% methanol) fractions.

The knowledge gained from this study will be helpful in isolation and characterization of new biologically active compounds ,active compounds associated with the antifungal activity, identification of Minimum Inhibitory Concentration (MIC), environmental friendly fungicides and new drugs in future. Thecharacterization of the compoundcan be done byusingadvance spectroscopic techniques such as IR, UV and NMR.

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