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Screening for *in vitro* antifungal activity and qualitative phytochemical analysis of the leaf extract of *Jasminum angustifolium*

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ABSTRACT

Jasminum angustifolium is endemic to Sri Lanka and it was reported that the roots and leaves are of medicinal value. Roots are bitter, acrid and are useful for external application in ring worm and herpes and are recommended for ophthalmopathy, ulcerative stomatitis and wounds. The juice of the leaves is given as an emetic in cases of poisoning. Present study was carried out to evaluate the *in vitro* antifungal activity of sequentially extracted different solvent extracts of leaves of *Jasminum angustifolium* against plant pathogenic fungi such as *Aspergillus* spp., *Penicillium* spp., *Trichoderma* spp., *Fusarium* spp. and *Alternaria* spp. by the standard agar well diffusion method. Synthetic fungicide Dithane M-45 (Mancozeb) and solvents used for extraction as standard and controls respectively. Qualitative phytochemical analysis of crude extracts was also carried out for the presence of bioactive compounds using standard procedures. Results revealed that the *J. angustifolium* leaf extracts had antifungal activity against all tested fungi at least in one solvent. The standard mancozeb and the methanol leaf extract exhibited antifungal activity against all tested fungi where as controls did not inhibit the growth of fungi. But hexane and ethylacetate leaf extracts failed to inhibit the growth of *Alternaria* spp. and *Trichoderma* spp. respectively. Effect of ethylacetate leaf extract against *Penicillium* spp.(22,17 mm) and *Fusarium* spp.(20,15 mm) was significant than compared to the standard mancozeb after 48,72 hours of incubation period. Growth inhibition on *Trichoderma* spp.(18 mm) and *Alternaria* spp.(14 mm) was significant by methanol leaf extract than compared to the standard after 24 hours of incubation. *Fusarium* spp.(18 mm) growth was significantly inhibited by hexane leaf extract than the standard after 72 hours of incubation period. Methanol leaf extract had a significant effect on fungal growth when compared with the other two leaf extracts. Phytochemical analysis indicated that various types of phytochemicals were in sequentially extracted hexane, ethylacetate and methanol extracts. Saponins, phlobatanins, terpenoids and alkaloids were present in all three extracts but flavanoids were only absent all extracts. This study revealed that the leaf of *J. angustifolium* had an antifungal activity against some plant pathogenic fungi and the presence of various types of phytochemical compounds.

Key words: *Jasminum angustifolium* leaf, sequential extraction, antifungal activity, agar well diffusion method, phytochemistry

INTRODUCTION

Herbal medicine refers to using a plant's seeds, berries, roots, leaves, bark or flowers for medicinal purposes. Medicinal plants can be used as biological control agents against microorganisms due to the presence of phytochemical compounds [1]. *Jasminum angustifolium* is endemic to Sri Lanka which is a wiry small scandent shrub with glabrous stem, pubescent branches. Leaves simple, small, ovate, acute rounded at base and glabrous.

Roots and leaves are of medicinal value. The juice of the leaves is given as an emetic in cases of poisoning [9]. Phytopathogenic fungi cause severe losses in plants and crop production. Therefore it is necessary to develop control measures that are cheap, ecologically friendly and environmentally safe. Most of the studies were based on the antibacterial activity of *J. angustifolium* plant part extracts. But biomolecules with antifungal activity have also been found in plants [3]. So the present study was carried out to evaluate the *in vitro* antifungal activity of sequentially extracted solvent extracts of leaves of *J. angustifolium* against plant fungal pathogens.

MATERIALS AND METHODS

Collection of plant materials

Fresh leaves of *J. angustifolium* were collected from five different places in the Jaffna peninsula of Sri Lanka.

Preparation of plant extracts

The fresh leaves were air dried and were ground well into powder form. 100 g of powder was taken in stopped bottle. 200 ml of hexane was added and soaked for three days with intermittent shaking. After three days the supernatant was filtered through a Buchner funnel. This procedure was repeated thrice to ensure the complete separation of all the constituents which were dissolve in hexane and extracts were pooled together. The solvent in the extract was evaporator under reduced pressure and reduced temperature by using rotatory evaporator. Then the remaining sample was allowed to air dry and the sample of hexane crude was weighed. The sequential extraction was followed by using ethyl acetate and then by methanol as solvents[2].

Test Fungi

Plant fungal pathogens, *Aspergillus spp.*, *Penicillium spp.*, *Trichoderma spp.*, *Fusarium spp.* and *Alternaria spp.* were obtained from the culture collections of the Department of Botany, University of Jaffna, Sri Lanka. These fungal cultures were sub cultured on potato dextrose agar (PDA)medium and were maintained as slants in the refrigerator for the future use.

Antifungal assay

Preparation of saline water (0.85%NaCl solution)

0.85 g NaCl was weighted and it was dissolved in 100 ml distilled water in a volumetric flask. Then 9.0 ml of the saline water was transferred into Mac Cartney bottles and those bottles were sterilized by an autoclave.

Preparation of fungal spore suspension

A loopful of fungal spores was taken with the help of a sterile inoculating loop from the mature culture on PDAmedium and suspended into sterile saline water under aseptic conditions. Then it was stirred well and the concentration was determined by the Haemocytometer. Concentration of suspension was adjusted to 10^7 spores/ml by the dilution technique.

Preparation of test solution

Mancozeb (Dithane M-45) as a synthetic antifungal agent was prepared in 0.3 mg/150 μ l concentration as standard. The solvent used to prepare the crude was considered as control.

Agar well diffusion method

0.1ml of each fungal suspension was spread uniformly on the entire surface of PDA plate by using a sterile spreader. 8 mm diameter wells were made by using a sterile cork borer. 100 μ l of each extracts were administered into each well separately. Corresponding solvent and Mancozeb were used as control and standard respectively. Plates were incubated at room temperature for 3-5 days and zone of inhibition around the well was measured at various time intervals such as 24, 48, 72 and 96 hours. Each experiment was repeated five times and the mean value was taken [3,4].

Phytochemical analysis

Qualitative analysis were carried out on crude extracts using standard procedures to identify the following components [5, 6,7, 8].

Test for Tannins

About 0.01 g of the crude extract was boiled in 20 ml of water in a boiling tube. Few drops of 0.1% of FeCl₃ were added. Formation of brownish green or a blue black coloration indicated the presence of tannins.

Test for Saponins

About 0.01g of the crude extract was boiled in 20 ml of distilled water in a water bath. Then it was mixed with 5 ml of distilled water and it was shaken well. Stable persistent froth indicated the presence of saponins.

Test for Phlobatanins

About 0.01 g of the crude extract was boiled with 1% aqueous hydrochloric acid. A deposition of a red precipitate indicated the presence of phlobatanins.

Test for Flavanoids

About 0.01 g of the crude extract was dissolved in 2 ml of ethanol solvent. Con.HCl and Mg turnings were added. A yellow coloration in extract indicated the presence of flavanoids.

Test for Steroids

About 0.01g of the crude extract was dissolved in 2ml of ethanol solvent. 2ml of acetic anhydride and 2ml of con.H₂SO₄ were added. The colour change from violet to blue or green indicated the presence of steroids.

Test for Cardiac glycosides

0.01g of crude extract was dissolved in 2ml of ethanol and then 2ml of glacial acetic acid which contained one drop of FeCl₃ solution was added. This was underlayered with 1ml of con H₂SO₄. A brownish ring of the interface indicated the presence of cardiac glycosides.

Test for alkaloids

About 0.01 g of crude extract was dissolved in 2ml of ethanol and it was divided into two parts. Few drops of Wagner's reagent along the wall of the test tube were added to one part. Brownish red precipitate indicated the presence of alkaloids.

Few drops of Mayer's reagent were added to the other part. A creamy white precipitate observed in extract indicated the presence of alkaloids.

Test for Terpenoids

5ml of crude extract was treated with 2ml of CHCl₃ and 3ml of con H₂SO₄ by adding carefully to form a layer. A reddish brown colouration of interface indicated the presence of terpenoids.

RESULTS AND DISCUSSION

Results showed that the *J. angustifolium* leaf extracts had antifungal activity against all tested fungi at least in one solvent. The standard mancozeb and the methanol leaf extract exhibited antifungal activity against all fungi where as controls did not inhibit the growth of fungi tested. Three solvent leaf extracts of *J. angustifolium* showed antifungal activity against all fungi except hexane and ethylacetate leaf extracts failed to inhibit the growth of *Alternaria spp.* and *Trichoderma spp.* respectively. Mean diameter of clear zone decreased with increasing incubation period. After 24 hours of incubation, none of the extracts showed any inhibitory effect on fungal growth. Effect of ethylacetate leaf extract against *Penicillium spp.* and *Fusarium spp.* was significant than compared to the standard mancozeb after 48,72 hours of incubation period. Growth inhibition on *Trichoderma spp.* and *Alternaria spp.* was significant by methanol leaf extract than compared to the standard after 24 hours of incubation. *Fusarium spp.* growth was significantly inhibited by hexane leaf extract than the standard after 72 hours of incubation period. Methanol leaf extract had a significant effect on fungal growth when compared with the other two leaf extracts. Bioactive compounds were extracted from the plant material according to their polarity.

Previous study on antimicrobial activity of *J. angustifolium* stated that antimicrobial activity of methanol flower extract of *J. angustifolium* did not show any inhibition against *Bacillus spp.*, *E. coli*, *Klebsiella spp.*, *Yersinia spp.* and *Enterococcus spp.* [10]. However *J. officinale* ethanolic flower extract exhibited antibacterial activity on *Staphylococcus spp.* and *Propionibacterium spp.* [11] and the chloroform leaf extract of *J. grandiflorum*L. also

exhibited antibacterial activity against *Klebsiella pneumonia*, *Staphylococcus aureus*, *E.coli* and *Salmonella typhi*[12]. Study on antimicrobial activity of flower and whole plant of *J. officinale* showed that the whole plant methanol extract exhibited significant antifungal activity against *Candida albicans* and *Aspergillus niger*, where as DCM extracts of whole plant and flower showed moderate activity against these fungi [13]. Methanol leaf extracts of *J. grandiflorum* and *J.sambac* showed most significant inhibitory effect on the growth of *Alternaria sp.* which caused foot infections in cancer patients[14]. But in this study hexane , ethylacetate and methanol leaf extracts exhibited antifungal activity against the fungal pathogens tested, except ethylacetate on *Trichoderma spp.* and hexane on *Alternaria spp.*

Table 1 : Antifungal activity of *Jasminum angustifolium* leaf extracts

Fungus	Incubation Time (Hours)	Leaves extracts of <i>Jasminum angustifolium</i> Mean diameter of clear zone (mm)			Mancozeb (standard)
		Hexane	Ethyl acetate	Methanol	
<i>Aspergillus spp.</i>	24	-	-	-	-
	48	15	18	11	18
	72	10	16	11	18
	96	-	15	10	16
<i>Penicillium spp.</i>	24	-	-	-	-
	48	12	22	11	14
	72	10	17	10	12
	96	09	14	10	12
<i>Trichoderma spp.</i>	24	-	-	-	-
	48	13	-	18	14
	72	11	-	18	13
	96	11	-	14	11
<i>Fusarium spp.</i>	24	-	-	-	-
	48	-	-	11	15
	72	18	20	10	14
	96	15	15	10	12
<i>Alternaria spp.</i>	24	-	-	-	-
	48	-	09	14	11
	72	-	09	13	10
	96	-	-	10	09

- no clear zone : No clear zone was observed in controls

Table 2: Qualitative Phytochemical tests for chemical constituents

Test	Leave extracts of <i>Jasminum angustifolium</i>		
	Hexane	Ethylacetate	Methanol
Tannins	-	+	+
Saponins	+	+	+
Phlobatanins	+	+	+
Steroids	+	=	+
Flavanoids	=	=	=
Terpenoids	+	+	+
Alkaloids	+	+	+
Cardiac glycosides	+	-	+

Note: + present ; - absent

Phytochemical analysis indicated the presence of various types of phytochemicals in sequentially extracted hexane, ethylacetate and methanol extracts. Saponins, phlobatanins, terpenoids and alkaloids were present in all three extracts but flavanoids were only absent all extracts. Previous studies on preliminary phytochemical screening of leaves of *J. grandiflorum* showed that alkaloids, flavanoids, saponins, steroids, tannins were present in ethanol extract and alkaloids, flavanoids, glycosides, saponins were in chloroform water extract and saponins, steroids, tannins were in acetone extract and alkaloids was only present in chloroform extract[15]. Ethanol extract and aqueous extract of *J.sambac* leaves showed the presence of alkaloids, flavanoids, saponins, steroids, tannins, glycosides and tannins, saponins respectively[16]. Variation in the results of these compounds was determined by the plant type, plant parts and the mode of solvent extraction[17].

CONCLUSION

Different solvent extracts exhibited different degree of antifungal activity among tested plant pathogenic fungi. *J. angustifolium* showed antifungal activity against all fungi except hexane and ethylacetate leaf extracts failed to inhibit the growth of *Alternaria spp.* and *Trichoderma spp.* respectively. Effect of ethylacetate leaf extract against *Penicillium spp.* and *Fusarium spp.* was significant and the growth inhibition on *Trichoderma spp.* and *Alternaria spp.* was also significant by methanol leaf extract than compared to the standard. Methanol leaf extract had a significant effect on fungal growth when compared with the other two leaf extracts. Phytochemical analysis indicated the presence of various types of phytochemicals in sequentially extracted hexane, ethylacetate and methanol leaf extracts of *J. angustifolium*. This study revealed that the leaf of *J. angustifolium* had an antifungal activity against some plant pathogenic fungi. Further studies could be developed to purify antifungal compounds in *J. angustifolium*.

REFERENCES

- [1] R.N.Chopra, S.L.Nayar, I.C.Chopra; Glossary of Indian Medical Plants, CSIR Publications, New Delhi, **1992**, 50.
- [2] Jeyasakthy. S., Jeyadevan. J. P., Thavaranjit. A. C., Manoranjan. T., Srikanan. R. and Krishnapillai, N. Antifungal activity and qualitative phytochemical analysis of extracts obtained by sequential extraction of some medicinal plants in Jaffna peninsula. *Archives of Applied Science Research*. **2013**, 5(6). 214-221.
- [3] L. Prince, P.Prabakaran, *Asian Journal of Plant Science and Research*., **2011**, 1(1), 84-87.
- [4] A.M.Clark, and F.S. El-Ferally, *Journal of Pharmaceutical Sciences*., **1981**, 70. 951-952.
- [5] F.J.A. Matos; Introducaoa fitoquimica experimental. Imprensa Universitaria, Universidade Federaldo ceara, **1988**, Folrtaleza, 128.
- [6] A.E. Sofowara; Medical Plants and traditional medicine in Africa. Part 11, Pitman Press Ltd, London, **1982**, 128-146.
- [7] G.E.Treas, W.C.Evans; A text book of Pharmacognosy. 12th Edition, Bailliere Tinnal Ltd, London, **1989**, 333-337.
- [8] J.B.Harbone; Phytochemical methods. A guide to modern Techniques of plant analysis, Chapman and Hall, London, **1973**, 199-204.
- [9] V.P.K.Nambiar; Indian medicinal plants, A compendium of 500 species. Vol 3, Orient Longman Private Ltd, Chennai, India, **2004**, 342.
- [10] V. Ramya, , V.D. Dhayalan, S. Umamaheswari, *Journal of Chemical and Pharmaceutical Research* ., **2010**, 2(6), 86-91.
- [11] G. S. Kumar, *Tropical Journal of Pharmaceutical Research*., **2007**, 6(2), 717-723.
- [12] J.D.Bhosale, M.Khond, T.K.Mandal, R.S.Bendre ,R.Dabur, *World Applied Sciences Journal*., **2011**, 13(1), 47-51.
- [13] M. Hussain, H. Bakhsh, A.Aziz, A.Majeed, I.A. Khan, A.Majeed, U. Farooq, *Journal of Pharmacy and Alternative Medicine*., **2013**, 2(4), 33-43.
- [14] A.Mishra, A. Shrivastava, K. Jains, *International Jjournal of Pharm Tech Research*., **2010**, 2(2), 1165-1170.
- [15] T.R. Sekharan, M.S. Mohan, R. Venkatnarayanan, A. T. Thirupathi, *Research Journal of Pharmacognosy and Phytochemistry*., **2010**, 2(6), 438-440.
- [16] S. Sabharwal, S. Aggarwal, M. Vats, S. Sardana, *International Journal of Pharmacognosy and Phytochemical Research*., **2012**, 4(3), 146-150.
- [17] K. Sathyaa, T. Manoranjan, A.C. Thavaranjit, J.P. Jeyadevan, *Der pharma chemica*., **2015**, 7(6), 282-286.