Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(13):144-148
(http://derpharmachemica.com/archive.html)

Screening for *in vitro* Antifungal activity and Qualitative Phytochemical analysis of the root extract of *Jasminum angustifolium*

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ABSTRACT

Jasminum angustifolium is a species of Jasmine endemic to Sri Lanka. 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, leprosy, pruritus and wounds. Present study was carried out to evaluate the in vitro antifungal activity of sequentially extracted different solvent extracts of roots 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 showed that the antifungal activity was exhibited by three solvents root extracts of J. angustifolium against all fungi except hexane and methanol root extracts failed to inhibit the growth of Aspergillus spp .and Trichoderma spp. respectively. Growth of Penicillium spp.(15mm) was significantly inhibited by all three root extracts than compared to other fungi tested. Growth inhibition by hexane and ethylacetate root extracts was significantly higher than compared to methanol root extract on the growth of Fusarium spp.(12mm,13mm) and Alternaria spp.(12mm,11mm). Ethylacetate root extract of J. angustifolium showed a significant effect on fungal growth inhibition compared with the other two root extracts. Different types of phytochemicals were present in sequentially extracted hexane, ethylacetate and methanol root extracts. Tannins, saponins, phlobatanins and steroids were present but flavanoids and terpenoids were absent in all three extracts.

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

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INTRODUCTION

Jasminum angustifolium is a species of Jasmine endemic to Sri Lanka. It is called as wild Jasmine in English, Kattumalligai in tamil and Wal pichcha/ Samanpichcha in Sinhala. This flower and *Jasminum grandiflorum* play a central role in Buddhist and Hindu temple floral offerings and garlands. It 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. Roots are bitter, acrid and are useful for external application in ring worm and herpes and are recommended for ophthalmopathy, ulcerative stomatitis, leprosy, pruritus and wounds[1,2].

Medicinal plants can be used as biological control agents against microorganisms due to the presence of phytochemical compounds [3]. Biological control treatments are usually 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 [4]. It is important to study the activity of antifungal compounds to control the activity of fungi which can cause diseases in plants. So the present study was carried out to evaluate the *in vitro* antifungal activity of sequentially extracted solvent extracts of roots of *J. angustifolium* against plant fungal pathogens.

MATERIALS AND METHODS

Collection of plant materials

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

Preparation of plant extracts

The fresh roots 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[5].

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 PDA medium 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 10^7spores/ml by the dilution technique[6].

Preparation of test solution

Mancozeb (Dithane M-45) as a synthetic antifungal agent was prepared in 0.3 mg/150µl 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. 100µl 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 [4,6,7].

Phytochemical analysis

Qualitative analysis were carried out on crude extracts using standard procedures to identify the following components [8,9,10,11].

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 $FeCl_3$ 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 shacked well. Stable persistent forth 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.H_2SO_4 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 $FeCl_3$ solution was added. This was underplayed with 1ml of con H_2SO_4. 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 $CHCl_3$ and 3ml of con H_2SO_4 by adding carefully to from a layer. A reddish brown colouration of interface indicated the presence of terpenoids.

RESULTS AND DISSCUSION

Results showed that the root extracts of *J. angustifolium* had antifungal activity against all tested fungi at least in one solvent. The standard antifungal agent Mancozeb exhibited the highest degree of activity against fungi where as controls did not inhibit the fungal growth. Antifungal activity was exhibited by three solvents root extracts of *J. angustifolium* against all fungi except hexane and methanol root extracts failed to inhibit the growth of *Aspergillus spp* .and *Trichoderma spp.* respectively. After 24 hours of incubation, none of the extracts showed any inhibitory effect on fungal growth. Mean diameter of clear zone decreased with increasing incubation period. Growth of *Penicillium spp.* was significantly inhibited by all three root extracts than compared to other fungi tested. Growth inhibition by hexane and ethylacetate root extracts was significantly higher than compared to methanol root extract on the growth of *Fusarium spp.* and *Alternaria spp.* Growth inhibition of *Aspergillus spp.* was significantly low by three root extracts than compared to other fungi tested. Ethylacetate root extract of *J. angustifolium* showed a significant effect on fungal growth inhibition compared with the other two root extracts.

]Previous 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 [12]. 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[13]. Hexane and ethylaceate leaf extracts of *J. angustifolium* did not inhibit

the growth of *Alternaria spp.* and *Trichoderma spp.* respectively. Methanol leaf extract of *J. angustifolium* had a significant effect on the growth of plant fungal pathogens, *Aspergillus spp., Penicillium spp., Trichoderma spp., Fusarium spp. and Alternaria spp.* when compared with the hexane and ethylacetate leaf extracts[6]. But in this study ethylacetate root extract of *J. angustifolium* showed a significant effect on the growth inhibition of *Aspergillus spp., Penicillium spp., Trichoderma spp., Fusarium spp. and Alternaria spp.* compared with the hexane and methanol root extracts.

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

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

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

Table 2: Qualitative Phytochemical tests for chemical constituents.

Test	**Root extracts of *Jasminum angustifolium***		
	Hexane	**Ethylacetate**	**Methanol**
Tannins	+	+	+
Saponins	+	+	+
Phlobatanins	+	+	+
Steroids	+	+	+
Flavanoids	_	_	_
Terpenoids	-	-	-
Alkaloids	+	-	-
Cardiac glycosides	-	+	-

Note: + present ; - absent

Different types of phytochemicals were present in sequentially extracted hexane, ethylacetate and methanol root extracts. Tannins, saponins, phlobatanins and steroids were present in all three extracts but flavanoids and terpenoids were absent in all three extracts. Previous studies on sequentially extraction of the hexane ,ethylacetate and methanol leaf extracts of *Jasminum angustifolium* showed that saponins, phlobatanins, terpenoids and alkaloids were present in all three extracts but flavanoids were only absent all extracts[6]. 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[14]. Variation in the results of these compounds was determined by the plant type, plant parts and the mode of solvent extraction [15].

CONCLUSION

Results showed that the root extracts of *J. angustifolium* had antifungal activity against all tested fungi at least in one solvent. Antifungal activity was exhibited by three solvents root extracts of *J. angustifolium* against all fungi except hexane and methanol root extracts failed to inhibit the growth of *Aspergillus spp* .and *Trichoderma spp.*

respectively. Ethylacetate root extract of *J. angustifolium* showed a significant effect on fungal growth inhibition compared with the other two root extracts. Different types of phytochemicals were present in sequentially extracted hexane, ethylacetate and methanol root extracts. Tannins, saponins, phlobatanins and steroids were present but flavanoids and terpenoids were absent in all three extracts. This study revealed that the root of *J. angustifolium* had an antifungal activity and further studies could be developed to purify these bioactive compounds.

REFERENCES

[1] V.P.K.Nambiar; Indian medicinal plants, A compendium of 500 species.Vol 3,Orient Longman Private Ltd,Chennai,India,**2004**,342.
[2] M.C.Joshi, A.Raju, A.Arulanandham, G.R.Saraswathy, *Pharmacologyonline*.,2008,3,197-205.
[3] R.N.Chopra, S.L.Nayar, I.C.Chopra; Glossary of Indian Medical Plants, CSIR Publications, New Delhi,**1992**,50.
[4] L. Prince, P.Prabakaran, *Asian Journal of Plant Science and Research*., **2011**, 1(1), 84-87.
[5] Jeyasakthy. S., Jeyadevan. J. P., Thavaranjit. A. C., Manoranjan. T., Srikaran. 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.
[6] K. Sathyaa, T. Manoranjan, A.C. Thavaranjit, *Der pharmacia Lettre*, **2015**,7(11), 147-151.
[7] A.M.Clark, and F.S. El-Feraly, *Journal of Pharmaceutical Sciences*., **1981**, 70. 951-952.
[8] F.J.A. Matos; Introducaoa fitoquimica experimental. Imprensa Universitaria, Universidade Federaldo ceara,**1988**, Folrtaleza,128.
[9] A.E. Sofowara; Medical Plants and traditional medicine in Africa. Part 11, Pitman Press Ltd, London, **1982**,128-146.
[10] G.E.Treas, W.C.Evans; A text book of Pharmacognosy. 12th Edition, Bailliere Tinall Ltd, London,**1989**, 333-337.
[11] J.B.Harbone; Phytochemical methods. A guide to modern Techniques of plant analysis, Chapman and Hall, London, **1973**,199-204.
[12] M. Hussain, H. Bakhsh, A.Aziz, A.Majeed, I.A. Khan, A.Majeeb, U. Farooq, *Journal of Pharmacy and Alternative Medicine*., **2013**, 2(4), 33-43.
[13] A.Mishra, A. Shrivastava, K. Jains, *International Jjournal of Pharm Tech Research*., **2010**, 2(2), 1165-1170.
[14] T.R. Sekharan, M.S. Mohan, R. Venkatnarayanan, A. T. Thirupathi, *Research Journal of Pharmacognosy and Phytochemistry*.,**2010**, 2(6), 438-440.
[15] K. Sathyaa, T. Manoranjan, A.C. Thavaranjit, J.P. Jeyadevan, *Der pharma chemica*., **2015**,7(6), 282-286.