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Research Article

IN VITRO ANTIFUNGAL ACTIVITY OF LEAF AND STEM BARK EXTRACTS OF THE ENDANGERED TRADITIONAL MEDICINAL TREE SPECIES, *HILDEGARDIA POPULIFOLIA* (ROXB.) SCHOTT & ENDL.

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ABSTRACT

Objective: The present study describes the antifungal activity of various alcoholic extracts of leaf and stem bark parts of *Hildegardia populifolia* (Sterculiaceae) and to determine minimum inhibitory concentration against certain fungal pathogens.

Methods: Various extracts of leaf and stem bark were assessed for antifungal activity against the species, *Aspergillus fumigatus, A. niger*, *Candida albicans, Paecilomyces lilacinus, Trichoderma viride, Mucor* sp., *Fusarium* sp. and *Penicillium* sp. by disc diffusion method. The minimum inhibitory concentration (MIC) was also determined. Ampilcillin was used as standard.

Results: Methanolic extract had highest antifungal activity against the tested fungi than the other solvent extracts. Further, by using most susceptible fungi, the MIC determined was 400µg/mL.

Conclusion: These results support that the leaf and stem bark of *H. populifolia* have prominent antifungal activity and it can be used to cure mycotic infections.

Keywords: *Hildegardia populifolia,* Sterculiaceae, Antifungal activity, MIC.

INTRODUCTION

In humans the fungal infections have increased severely in recent years. Microorganisms have developed resistance to many antibiotics and as a result, immense clinical problem in the treatment of infectious diseases has been created. Due to the increasing development of drug resistance in human pathogens as well as the appearance of undesirable effect of certain antimicrobial agents, there is a need to search for new antifungal agents without toxicity and side effects [1]. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. In many developing countries, treatment with traditional medicine is one of the primary healthcare systems [2,3]. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine [4].

Hildegardia populifolia is an indigenous endangered medicinal tree species belongs to the family, Sterculiaceae. It is distributed in dry deciduous forests of Tamilnadu and Andrapradesh. The plant extract is mainly prescribed for the treatment of malaria and dog bite by traditional healers [5]. It is reported to have the properties of antibacterial activity [6,7], antioxidant [8] and antiinflammatory also [9]. However, no studies are made for antifungal properties of this species. To address this lacuna, the present study on antifungal property was carried out by using various alcoholic extracts of leaf and stem bark parts of the species, *H. populifolia.* The minimum inhibitory concentration (MIC) against certain pathogenic fungi was also determined.

MATERIALS AND METHODS

Collection and identification of plant material

The leaf and stem bark of *H. populifolia* was collected from the campus of Forest Genetics Division, Bhavanisagar, Tamil Nadu, India. The plant was identified and confirmed by a voucher specimen with authentic specimen (15211 (MH)) deposited in the herbarium at Botanical Survey of India, Southern Circle, Coimbatore, India.

Preparation of plant extract

The shade dried leaves and stem bark of the study species were separately made into fine powder of 40 mesh size using the pulverizer. Following that, 100g of the powder was filled in the filter paper and successively extracted using 500 mL solvents *viz*., petroleum ether, hexane, chloroform and finally with methanol using the soxhlet extractor for 8 – 10 hours [10]. The extract was filtered through Whatman No.1 filter paper to remove all undissolved matter, including cellular materials and other constitutions that are insoluble in the extraction solvents.

Fungal strains

Aspergillus fumigatus, A. niger, Candida albicans, Paecilomyces lilacinus, Trichoderma viride, Verticillium lecanii, Mucor sp. *Fusarium* sp. and *Penicillium* sp. were obtained from the Department of Microbiology, Hindustan College of Arts and Science, Coimbatore. The fungal strains were maintained at 4ºC on potato dextrose agar slants and kept in refrigerator prior to subculture.

Media used

Freshly prepared Potato Dextrose Agar medium was used for the culture of fungi.

Antifungal activity

Disc diffusion method

The culture media were prepared and autoclaved at 121ºC at 15 p.s.i. for 20 minutes and stored in refrigerator. The media were melted before the process of inoculation. The clean dry sterile Petri dishes were poured with potato dextrose agar medium. Ten numbers of 10 mL broths were prepared separately for potato dextrose agar medium in test tubes and plugged with cotton and autoclaved. The test tubes were labeled with the microbes to be inoculated. The fungal strains were inoculated onto potato dextrose broth under aseptic conditions and incubated at 37 ± 0.5ºC for 18 hrs. After incubation, the fungi were smeared on the potato dextrose agar plate separately using a sterile cotton swab. A sterile disc of 6 mm diameter was loaded with known quantity of 10 mg of dried crude extracts. These discs were placed on the surface of the media. Ampicillin antibiotic disc was used as positive control. Then the Petri dishes were incubated at 37±0.5 ºC for 24 to 48 hrs. The diameter of inhibition zone was measured. Triplicates were maintained for all tests [11].

Determination of Minimum Inhibitory Concentration (MIC)

Based on the effective performance of methanolic extract, it was analysed for further studies on minimum inhibitory concentration (MIC).

Test cultures used

Trichoderma viride, Verticillium lecanii, Mucor sp., *Aspergillus niger, Fusarium* sp. and *Penicillium* sp. cultures were used for assaying minimum inhibitory concentration of antifungal activity of methanolic leaf and stem bark extracts of *Hildegardia populifolia.*

Method

The minimum inhibitory concentration was determined through the microbroth dilution method [12]. Test tubes with 1800µL of potato dextrose broth were taken separately for fungal strain. Different concentrations of methanolic leaf and stem bark extracts ranged from 100 to 800 µg/mL were incorporated into the broth and the tubes were then inoculated with 200 µL of inoculum of fungi which was diluted for five times (10^{-5}dilution) to control its vigorous growth and 200 µL of inoculum was added into the respective fungal broth and kept at 37°C for 24-48 hrs. The test tube containing the lowest concentration of extract which showed reduction in turbidity, when compared with positive control, ampicillin and negative control (DMSO) was regarded as MIC of that extract.

Statistical analysis

The results were expressed as mean±SD. The data were subjected to one way analysis of variance (ANOVA) and the significance between mean was determined by Duncan's Multiple Range test with significance level, P<0.05. ANOVA was performed using the statistical software SPSS (SPSS Inc. Chicago, USA).

RESULTS

Antifungal activity

The results of antifungal studies of leaf and stem bark parts of the study species, *Hildegardia populifolia* are presented in Tables 1 and 2. Generally, the methanolic extracts of both parts were determined to have greater inhibitory effect than that of the other alcoholic solvents. The methanolic leaf and stem bark extracts respectively registered higher zone of as 22 and 25 mm diameter of inhibition against the fungus, *Trichoderma viride* followed by another fungus, *Verticillium lecanii* (21 and 20 mm inhibition zone diameter by leaf and stem bark parts respectively). It was further observed that the inhibitory activity of methanol extracts of both leaf and stem bark against the fungi, *Aspergillus niger* and *Mucor* sp. was determined to be most noteworthy.

Table 1: Antifungal activity of certain alcoholic leaf extracts of *Hildegardia populifolia.*

Leaf extract	Diameter of zone of inhibition (mm)								
	Aspergillus fumigatus	***A. niger***	***Candida albicans***	***Paecilomyces lilacinus***	***Trichoderma viride***	***Verticillium lecanii***	***Mucor* sp.**	***Fusarium* sp.**	***Penicillium* sp.**
Standard*	20^{a}±0.12	25^{a}±0.08	13^{a}±0.35	26^{a}±0.54	30^{a}±0.71	20^{a}±0.20	21^{a}±0.08	19^{a}±0.24	15^{a}±0.51
Petroleum ether	-	9^{d}±0.34	-	-	8^{e}±0.52	6^{d}±0.24	11^{c}±0.26	8^{d}±0.36	7^{c}±0.64
Hexane	-	8^{de}±0.06	-	9^{cd}±0.28	10^{d}±0.12	10^{c}±0.36	10^{d}±0.51	-	-
Chloroform	10^{bc}±0.05	11^{c}±0.15	-	10^{c}±0.31	15^{c}±0.34	13^{b}±.0.13	11^{c}±0.43	10^{c}±0.35	12^{b}±0.37
Methanol	11^{b}±0.24	17 ^{b}±0.27	10^{b}±0.07	15^{b}±0.34	22^{b}±0.25	20^{a}±0.41	16^{b}±0.17	14^{b}±0.28	15^{a}±0.32

*Ampicillin

Values are expressed as mean±SD (n=3).

Values within the same column not sharing common superscript letters (a-e) differ significantly at p<0.05 by DMRT.

Table 2: Antifungal activity of certain alcoholic stem bark extracts of *Hildegardia populifolia.*

Stem bark extract	Diameter of zone of inhibition (mm)								
	Aspergillus fumigatus	***A. niger***	***Candida albicans***	***Paecilomyces lilacinus***	***Trichoderma viride***	***Verticillium lecanii***	***Mucor* sp.**	***Fusarium* sp.**	***Penicillium* sp.**
Standard*	23^{a}±0.27	15^{b}±0.53	12^{a}±0.34	21^{a}±0.81	30^{a}±0.06	25^{a}±0.05	22^{a}±0.54	16^{a}±0.34	20^{a}±0.15
Petroleum ether	-	9^{de}±0.64	-	9^{d}±0.61	10^{e}±0.13	11^{d}±0.13	14^{de}±0.67	6^{de}±0.25	6^{de}±0.38
Hexane	9^{d}±0.13	10^{d}±0.50	-	-	15^{d}±0.40	9^{e}±0.12	11^{e}±0.04	7^{d}±0.16	7^{d}±0.09
Chloroform	10^{c}±0.26	12^{c}±0.27	7^{c}±0.33	12^{c}±0.34	22^{c}±0.52	16^{c}±0.55	15^{c}±0.11	13^{c}±0.34	8^{c}±0.46
Methanol	12^{b}±0.08	18^{a}±0.21	10^{b}±0.15	12^{b}±0.34	25^{b}±0.83	21^{b}±0.41	20^{b}±0.55	19^{b}±0.25	14^{b}±0.30

* Ampicillin

Values are expressed as mean±SD (n=3).

Values within the same column not sharing common superscript letters (a-e) differ significantly at p<0.05 by DMRT.

Table 3: The minimal inhibitory concentration (MIC) of methanolic leaf and stem bark extracts of *Hildegardia populifolia* on certain pathogenic fungi.

Organisms	Leaf extract (µg/mL)									Stem bark extract (µg/mL)								
	50	100	200	300	400	500	600	700	800	50	100	200	300	400	500	600	700	800
*Standard	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
richoderma viride	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+
Verticillium lecanii	-	-	-	-	-	+	+	+	+	-	-	-	-	-	+	+	+	+
Mucor sp.	-	-	-	-	-	+	+	+	+	-	-	-	-	-	+	+	+	+
Aspergillus niger	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	+	+	+
Fusarium sp.	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+
Penicillium sp.	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+
**Negative control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(+) - activity; (-) – no activity

* Ampicillin; ** DMSO – (Dimethyl Sulfoxide)

Minimum inhibitory concentration (MIC) assay

Minimum inhibitory concentration (MIC) values of the extract against the tested fungi are shown in Table 3. The methanolic extract of both leaf and stem bark parts of *Hildegardia populifolia* generally showed significant inhibitory activity against all the fungal species with the MIC being 500 µg/mL. However, the MIC of methanolic extracts of both parts was fungal species-specific also. The leaf extract recorded the MIC value 400 µg/mL against the fungi, *Trichoderma viride, Aspergillus niger, Fusarium* sp. and *Penicillium* sp. and the stem bark of that was recorded as 400 µg/mL against the fungi, *Trichoderma viride, Fusarium* sp. and *Penicillium* sp.

DISCUSSION

It is important to investigate scientifically about the plants that have been used in traditional medicines to determine potential sources of novel antimicrobial compound [13]. The results of the present study on the antifungal activities of various organic solvent extracts of the leaf and stem bark parts of the study species, *Hildegardia populifolia* showed that they have potent inhibitory effects against the tested fungal species (Tables 1and 2). Further it was found that generally the inhibitory activity of *H. populifolia* is pathogen-specific and depends on the solvent type and plant part used for the extraction. The leaf and stem bark of this species were effectively suppressed the mycelial growth of fungi in methanol extract followed by chloroform extract. Similar trend of results were obtained by Astiti and Suprapta, [14] who observed that the methanol extract of Teak leaf obviously inhibited the growth, spore formation and biomass production of the fungus, *Arthrinium phaeospermum.* In the same family, Sterculiaceae, various other plant species have also been reported for better inhibitory activity against many pathogenic fungi [15, 16]. Petroleum ether and hexane extracts had lower activity. It is explained that the less amount of bioactive compounds extracted by these low polar solvents may not be adequate qualitatively for the interaction with the fungi to check their growth. Differences in polarity among various solvents have been reported already to account for the differences in solubility of plant active properties, hence variations in the degree of activity [17].

From the study it is known that the inhibitory effect of the leaf and stem bark extracts of *Hildegardia populifolia* against the pathogenic fungal species *viz., Trichoderma viride*, *Verticillium lecanii*, *Mucor* sp., *Aspergillus niger, Penicillium* sp. and *Fusarium* sp. was more prominent. Generally, the methanolic extract of leaf and stem bark parts of *Hildegardia populifolia* at the concentration of 400 and 500 µg/mL respectively were determined to be most effective against the tested fungal organisms. This may be attributed to the presence of soluble phenolics and polyphenolic compounds reported in this species [18,19] which perhaps served as antifungal agents. *Trichoderma viride, Penicillium* sp. and *Fusarium* sp. were more susceptible fungi for both leaf and stem bark methanolic extracts in the concentration of 400µg/mL. The results of the study revealed that antifungal activity of the crude extracts was enhanced by increasing the concentration of the extracts. This finding is in agreement with the report of Dellavalle *et al*. [17], who also observed that the increasing of concentrations of antimicrobial substances exhibited more inhibitory activity on growth of fungi. It is proved that both the extract possesses more fungicidal action against the above mentioned fungi. Plants rely on other mechanisms include synthesis of bioactive organic compounds [20] and antifungal proteins [21] and peptides [22] to defend themselves from infection by a variety of pathogens. The quantity and quality of these active compounds depend on the plant species, their parts and environmental factors [23,24].

In conclusion it is to be stated that the leaf and stem bark parts of the study species *Hildegardia populifolia* can be used as potential sources for drug development for the treatment of ailments caused by tested fungal pathogens. However, further *in vitro* studies are required to confirm the property of antifungal activity.

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