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The Biological Screening of Extracts/Fractions of Various Parts of *Pistacia integerrima* Stewart against Pathogenic Fungi

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Abstract

The goal of the current study was to scrutinize the antifungal activity of the crude extracts and subsequent solvent fractions of different parts of *Pistacia integerrima* against selected pathogenic fungi using agar tube dilution method. The results illustrated susceptibility of extracts/fractions of different parts of the plant against *Microsporum canis, Aspergillus flavus and Fusarium solani.* However, the rest of fungi were not sensitive to extracts/fractions. In conclusions, our study provided a strong evidence for the isolation of secondary metabolites from different parts of the plant as antifungal.

Keywords: Pistacia integerrima; Extracts/fractions of different parts; Antifungal activity

Introduction

Pistacia integerrima (J. L. Stewart ex Brandis) belongs to family anacardiacea. It mostly grows at a height of 12000 to 8000 feet in Eastern Himalayan regions [1]. It is a variable sized tree that can grow up to forty feet. *P. integerrima* has been used in the treatment of inflammation, diabetes, blood purification, gastrointestinal problems, and as an expectorant. Indian are using this plant as antiasthmatic, antipyretic, antiemetic and antidiarrheal [2,3]. In Pakistan, galls of *P. integerrima* are used for the treatment of hepatitis and other liver disorders [4,5], infections, diabetes, pain, inflammatory conditions, and fever [6].

Phytochemically, monoterpenes, triterpenoids sterols, dihydromalvalic acid and flavonoids have been isolated from the different parts of *Pistacia* species [7,8]. In this research article, we present the experimental findings of various fractions of different parts of *P. integerrima* against selected pathogenic fungi.

Material and Methods

Collection of plant materials

P. integerrima were collected from Toormang, Razagram (District Dir), Khyber Pakhtunkhwa, Pakistan in the month of February, 2010. The identification of plant material was done by Prof. Dr. Abdur Rashid plant taxonomist Department of Botany, University of Peshawar. A voucher specimen (Bot.20037(PUP) was deposited in the herbarium of the same institution.

Extraction and fractionation

Shade dried and crushed galls, leaves, bark and roots of *P. integerrima* (Stewart) were extraction with ethanol. The resulting methanolic extract of each part was suspended in water and sequentially fractionated with n-hexane, chloroform and ethyl acetate in order to get respective major fraction.

Fungal strains

Fungal strains include *Trichyton logifusus (clinical isolate), Candida albicans* ATCC 2091, *Candida glabrata* ATCC 90030 *Aspergillus flavus* ATCC 32611*, Microsporum canis* ATCC 11622, and Fusarium solani 11712. They were maintained on agar slant at 4°C. Before any test, the activation of strains was occurred at 37°C for 24 h on Sabouraud Glucose Agar (SGA).

Antifungal assay

Agar tube dilution method was employed for the assessment of antifungal activity of extracts and fractions of various parts *P. integerrima* [9]. The extracts as well as fractions (24 mg/ ml) were dissolved in the sterile Dimethyl sulfoxide (DMSO) for the preparation of stock solution. Sabouraud Dextrose Agar (SDA) 4 ml was dispensed into screw cap tubes, which were autoclaved at 120°C for 15 min and then cooled to 15°C. The non-solidified SDA media was poisoned with stock solution (66.6 µl) giving the final concentration of 400 µg of the extract per ml of SDA. Tubes were allowed to solidify in the slanted position at room temperature. Each tube was inoculated with a piece (4 mm diameter) of inoculums removed from a seven days old culture of fungi for non-mycelial growth; an agar surface streak was employed. DMSO was used as control while miconazol and amphotericin-B as standard drugs. Inhibition of fungal growth was observed after 7 days of incubation at 28 ± 1°C and relative humidity (40-50%). The test tubes were analyzed for the visible growth of the microorganisms and % antifungal activity was determined.

Statistical analysis

The experimental findings are mean ± SEM of three different readings. Analysis was performed by using GraphPad Prism version 6.

Results

Antifungal effect of the galls *P. integerrima*

The antifungal activity of the methanolic extract and subsequent solvent fractions of the galls of *P. integerrima* are presented in Table 1. Of the test fungi, *M. canis* was the only susceptible fungus to various extracts and fractions of the galls of the plant. However, the rest of fungi were not receptive to extract and fractions.

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Antifungal effect of the leaves of *P. integerrima*

The results of antifungal methanolic extract and subsequent solvent fractions of the leaves of *P. integerrima* are presented in Table 2. *M. canis, A. flavus* and *F. solani* evoked sensitive to extract/fractions among the test fungi.

Antifungal effect of the bark of *P. integerrima*

The effects of test extract/fractions of the bark of *P. integerrima* are presented in Table 3. The results showed susceptibility of extract/fractions only against *M. canis and A. flavus*.

Antifungal effect of the roots of *P. integerrima*

The antifungal effects of the roots of *P. integerrima* against selected pathogenic fungi are illustrated in Table 4. The extract/fractions exhibited activity only against *M. canis* and *A. flavus*.

Discussion

The current article was designed to evaluate the antifungal activity of extracts/fractions of various parts (galls, leaves, bark and roots) of *P. integerrima* against various pathogenic fungi.

In the course of time, dramatic changes have been observed in human mycoses. Along with a significant increased in the number of infected patients, there have been noted changes in the fungal pathogens. The best possible explanation for these alterations is both the emergence of new fungal pathogens and the expanding spectrum with more serious infections due to classic fungal pathogens. Probably, these effects are paralleled and directly related to increases in patient populations that are highly susceptible to fungal infections. These effects mostly including patients with surgery, cancers, bone marrow and solid organ transplant recipients, and other immunosuppressed patients, most notably those infected with the Human-Immune-

Name of Fungus	**% Zone of inhibition (mm)**					
	n-Hexane	Chloroform	Ethyl acetate	Methanol	STD	MIC (µg/ml)
Trichophyton longifusus	-	-	-	-	Miconazole	
Candida albicans	-	-	-	-	Miconazole	110.8
Aspergillus flavus	-	20 ± 0.50	-	-	Amphotericin B	20.20
Microsporum canis	20 ± 0.42	35 ± 0.46	25 ± 0.57	15 ± 0.21	Miconazole	98.4
Fusarium solani	-	-	-	-	Miconazole	73.25
Candida glabrata	-	-	-	-	Miconazole	110.8

Results represent the mean of three different experimental readings

Table 1: Antifungal (%) extract and fractions of the galls of *P. integerrima* against test fungi

Name of Fungus	**% Zone of inhibition (mm)**					
	n-Hexane	Chloroform	Ethyl acetate	Methanol	STD	MIC (µg/ml)
Trichophyton longifusus	-	-	-	-	Miconazole	
Candida albicans	-	-	-	-	Miconazole	110.8
Aspergillus flavus	20 ± 0.22	25 ± 0.53	-	20 ± 0.23	Amphotericin B	20.20
Microsporum canis	-	30 ± 0.68	30 ± 0.63	20 ± 0.12	Miconazole	98.4
Fusarium solani	20 ± 0.34	-	20 ± 0.37	-	Miconazole	73.25
Candida glabrata	-	-	-	-	Miconazole	110.8

Results represent the mean of three different experimental readings

Table 2: Antifungal (%) extract and fractions of the leaves of *P. integerrima* against test fungi

Name of Fungus	**% Zone of inhibition (mm)**					
	n-Hexane	Chloroform	Ethyl acetate	Methanol	STD	MIC (µg/ml)
Trichophyton longifusus	-	-	-	-	Miconazole	
Candida albicans	-	-	-	-	Miconazole	110.8
Aspergillus flavus	-	20 ± 0.02	-	-	Amphotericin B	20.20
Microsporum canis	20 ± 0.21	35 ± 0.49	25 ± 0.72	15 ± 0.03	Miconazole	98.4
Fusarium solani	-	-	-	-	Miconazole	73.25
Candida glabrata	-	-	-	-	Miconazole	110.8

Results represent the mean of three different experimental readings

Table 3: Antifungal (%) extract and fractions of the barks of P. integerrima against test fungi

Name of Fungus	**% Zone of inhibition (mm)**					
	n-Hexane	Chloroform	Ethyl acetate	Methanol	STD	MIC (µg/ml)
Trichophyton longi-fusus	-	-	-	-	Miconazole	
Candida albicans	-	-	-	-	Miconazole	110.8
Aspergillus flavus	-	20 ± 0.32	-	-	Amphotericin B	20.20
Microsporum canis	20 ± 0.08	30 ± 0.63	25 ± 0.47	20 ± 0.19	Miconazole	98.4
Fusarium solani	-	-	-	-	Miconazole	73.25
Candida glabrata	-	-	-	-	Miconazole	110.8

Results represent the mean ± SEM of three different experimental readings

Table 4: Antifungal (%) extract and fractions of the roots of P. integerrima against test fungi

Deficiency Virus (HIV) [10].

M. canis is involved in the pathogenesis of tinea capitis in humans, and simple ringworm in animals (pets). The organism's major reservoir in companion animals is within domestic cats and dogs. Under Wood's lamp examination, it gives bright green fluorescence. It is closely related to other dermatophytes [11]. The extracts/fractions of different parts of the plant showed antifungal activity against *M. canis.* It is therefore, suggested that the extracts/fractions of different parts of the plant possessed active constituents that were sensitive to M. canis.

A. flavus is a common filamentous fungus that produces aflatoxins and presents a major threat to agriculture and human health [12]. The extracts of different parts of the test plant illustrated some activity against A. flavus. The results suggested the presence of pharmacologically active secondary metabolites that exhibited antifungal activity.

Members of the *F. solani* species are increasingly implicated as the causative agents of human mycoses, particularly in the expanding immunocompromised and immunosuppressed patient populations [13]. Based on our finding, extracts possessed active molecules that had some antifungal potential.

In short, the present study demonstrated wide antifungal sensitive of extracts/fractions of *P. integerrima* against different pathogenic fungi. Isolation of secondary metabolites from different parts of the plant will explain the chemical background of current study.

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