Research Article

Antifungal activity of crude extracts of stem-bark of *Acacia ampliceps* Maslin. (Family Leguminosae)

Fatima*, Muhammad Saleem Khan, Maqsood Anwar, Sidra Rahman and Muhammad Anwar Sajad

Department of Botany, Islamia College Peshawar-Pakistan

*Corresponding author's email: fatimaimtiaz535@gmail.com

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Abstract

The present study explains antifungal activity of crude extracts of stem-bark of *Acacia ampliceps* Maslin (Family Leguminosae). Two different extracts of the stem-bark of this plant were used against four fungal strains. It was revealed that methanol extract was comparatively inhibitorier to all fungal strains than ethanol. Methanol extract showed significant inhibition against *Trichoderma spp.*, fair inhibition against *Rhizopus* and *Acremonium spp.* and less activity against *Aspergillus niger*. However, ethanol extract showed comparatively high activity against *Trichoderma spp.* while the remaining three fungal strains were comparatively resistant to it. Maximum zone of inhibition was recorded to be 22 mm by methanol extract at 1000 µg/mL against *Trichoderma spp.*

Keywords: *Acacia ampliceps*; Crude extract; Fungal strains; Medicinal plants; Zone of inhibition

Introduction

Acacia ampliceps belongs to family Mimosaceae (Leguminosae) is a shrub of 2-8 m height. It has 1-4 stems branches arise from the ground level having yellow color twigs with yellowish-green or brown-green, spreading canopy. It is native to the Northern parts of Western Australia. The plant is cultivated in different parts of Pakistan for medicinal, ornamental and economic purposes. Medicinal plants (MP) are traditionally used for the treatment of various human ailments. In different countries, plants are used as prospective source of antimicrobial agents [1]. About 60 to 90% populations of the developing countries use

plant-derived medicines. Traditionally, crude plant extracts (CPE) are used as herbal medicine for the treatment of various human infectious diseases [1-3]. Medicinal plants are rich in a variety of phytochemicals such as alkaloids, glycosides, flavonoids, terpenoids, tannins and phenolic compounds which have been found *in vitro* to have antimicrobial properties [4-6].

Indeed plants possess a wide variety of bioactive molecules, which make them a rich home of different brands of medicines. Higher plants, as sources for remedial compounds, have sustained to perform a leading role in the maintenance of human health since ancient times [7]. *Acacia* spp.

possess secondary metabolites like alkaloids, cyanogenic glycosides, amines, terpenes, saponins, hydrolysable tannins, condensed tannins, flavonoids, cyclitols, fluoroacetate, non-protein amino acids, gums, fatty acids, seed oils, essential oils, sterols and genins [8]. *Acacia ampliceps* is one of precious medicinal plant species which exhibit antimicrobial (antifungal) potential due to presence of variety of phytochemicals compounds in it. The present study aimed to investigate the antifungal activity of ethanol and methanol extract of *Acacia ampliceps* Maslin stem-bark against four fungal strains.

Materials and methods

Plant collection and identification

Disease free stem-bark of *Acacia ampliceps* was collected from the Pakistan Forest Institute, Peshawar. It was identified with the help of available literature and then confirmed [9]. After identification the plant is then deposited in herbarium of Botany department, Islamia College Peshawar for future references.

Preparation of crude extract

The stem-bark was sliced into small parts and thoroughly washed with tap water to remove dust and dirt. In order to avoid the evaporation of volatile oils and chemicals, the stem-bark was dried in shade for 15 to 25 days. The dried stem-bark was ground through mechanical grinder to get fine powder. 100 g powder was soaked in 600 mL methanol and in 600 mL ethanol for 21 days. Both solutions were filtered using Wattman filter paper No.1 in separate beakers and concentrated under reduced pressure at 45° C in rotary evaporator. Then both extracts were shifted to separate china dishes and further dried in Water bath at 55° C to obtain crude extracts. Finally methanol and ethanol crude extracts were stored in 18 g and 14 g viles respectively [10]. These extracts were preserved in refrigerator at 4° C to prevent contamination and processed for antifungal activity.

Antifungal activity

Both extracts were tested for its antimicrobial activity (antifungal) by agar well diffusion method [11, 12] with slight modification.

Preparation of stock solution and working dilution

For the preparation of stock solution 1000 µg/mL, 20mg of crude extracts (both methanol and ethanol) was dissolved in 2mL DMSO (Dimethyl sulfoxide) in a test tube and shaken well via Vortex mixture. This solution was then shifted to Eppendorf tube. Working solution (5 mg/mL) was made by mixing 500 µg/mL of stock solution with equal volume of DMSO.

Standard control

In case of antifungal bioassay, 0.05% Nystatin (25ml/cc and 75ml/cc DMSO) was used as positive control and 500 µg/mL DMSO was used as negative control.

Test fungal strains used

Four different strains of fungi were taken from the laboratory of Department of Botany Islamia College Peshawar. These were *Acremonium spp.*, *Aspergillus niger*, *Rhizopus spp.* and *Trichoderma spp.*

Media preparation

Potato Dextrose Agar (PDA) was used for culturing of fungal strains. About 9.75 g Potato Dextrose Agar (PDA) was dissolved in 300 ml of distilled water and then mixed with the help of Thermo-Magnetic Stirrer and poured in autoclavable bottle and sterilized in autoclave at 121° C for 15 minutes under high pressure.

Inoculation and antifungal bioassay

Fungal strains were streaked on media plates using sterile cotton swabs and then incubated for a day. Potato Dextrose Agar (PDA) was poured in two sets (one set for one extract) of four sterile petri dishes in laminar hood. After about 15 minutes, the media changed into a jelly-like substance. Four wells were bored via a sterile cork borer of 5mm at equal distance from each other. To these wells, 1000 µg/mL extract stock solution, 500

µg/mL working dilution, positive control and negative control were added separately via micropipette and zone of inhibition (ZOI) were measured by using ruler in millimeter (mm) in diameter around the wells from the bottom of the plates.

Results

Results revealed that methanol extract showed comparatively high activity against *Trichoderma* while rest of the three fungal strains were comparatively resistant to it. On the other hand, methanol extract significant inhibition against *Trichoderma spp.*, fair inhibition against *Rhizopus* and *Acremonium spp.* and less activity against *Aspergillus niger*. *Acremonium* was very sensitive to both ethanol and methanol extracts of *Acacia ampliceps* stem-bark in both concentrations i.e. 1000 µg/mL and 500 µg/mL. But methanol extract showed significant results than that of ethanol. It was noted that ZOI was directly proportional to the concentration of the extract applied i.e. high for 1000 µg/mL and low for 500 µg/mL. Zone of inhibition of standard drug Nystatin was higher than the extracts. At 1000 µg/mL, ethanol and methanol showed ZOI of 16mm and 20mm while ZOI at 500 µg/mL was recorded to be 12mm and 16mm respectively. Zone of inhibition of Nystatin was recorded to be 22mm. *Aspergillus niger* was very sensitive to both ethanol and methanol extracts of *Acacia ampliceps* stem-bark and showed the same results at both concentrations i.e. 1000 µg/mL and 500 µg/mL. Zone of inhibition of Nystatin was higher than that of the applied extracts at both concentrations. At 1000 µg/mL as well as 500 µg/mL, ethanol and methanol showed the same ZOI of 16mm and 14mm respectively. Zone of inhibition of 0.05% Nystatin was recorded to be 18mm. *Rhizopus spp.* was very sensitive to both ethanol and methanol extracts of *Acacia ampliceps* stem bark at both concentrations i.e. 1000 µg/mL and 500 µg/mL. Methanol showed significant results

than ethanol. Zone of inhibition of methanol at high concentration (1000 µg/mL) is the same as that of standard drug (Nystatin). Zone of inhibition at low concentration (500 µg/mL) of both extracts were same and lower than that of Nystatin. Methanol at 1000 µg/mL, exhibited a ZOI of 20mm which equal to that of Nystatin and that of ethanol is measured to 16mm. At 500 µg/mL, ethanol and methanol showed the same ZOI of 14mm. *Trichoderma spp.* was very sensitive to both ethanol and methanol extracts of *Acacia ampliceps* Maslin stem-bark at both concentrations i.e. 1000 µg/mL and 500 µg/mL. Zone of inhibition was directly proportional to the concentration of the extract applied i.e. high for 1000 µg/mL and low for 500 µg/mL. Methanol showed significant results than ethanol. Zone of inhibition of the standard drug (Nystatin) was higher than both extracts. Methanol at 1000 µg/mL, exhibited a ZOI of 20mm which equal to that of Nystatin and that of ethanol is measured to 16mm. At 500 µg/mL, ethanol and methanol showed the same ZOI of 14mm (Table 1; Fig. 1 & 2).

Discussion

Adversative side effects of antibiotics are known to cause some unusual serious infections, which enforced researchers to discover new cradles of effective antimicrobial medications. For this tenacity, plants have been analyzed for safe antimicrobial constituents which are proved to be a latent source of novel compounds of known constructive features [13-15]. A widespread array of therapeutic plant parts is used for extracts as raw drugs and they retain various medicinal physical characteristics. The different parts used are stem, flower, root, leaves, twigs exudates, fruit and modified plant organs. While some of these raw drugs are collected in lesser quantities by the native people and folk healers for indigenous used, many other raw drugs are collected in greater quantities and traded in

the market as the raw material for many herbal pharmaceuticals [16].

Herbal medicines are nowadays regarded as the greatest substitute of antibiotics with no or very less side effects [17]. The use of active phytochemicals, in combination with antibiotics could elude the advent of resistant variants that might otherwise arise during treatment. These blends are likely to exert a synergistic effect or to lessen likely adverse side effects [18]. Though numerous plant species have been tested for their antimicrobial potential, majority of them have not been professionally estimated [19]. Due to the presence of effective antimycotic agents, and the advent of resistant or multi-resistant strains, a permanent search and development of new drugs is desired [20]. The valuable remedial properties of plant materials normally result due to the secondary metabolites present in the plant although, it is usually not credited to a solo compound but a mix of different metabolites. The therapeutic actions of plants are unique to a specific plant species or group, consistent with the concept that the amalgamation of secondary products in a particular plant is taxonomically distinct [21].

A lot of work has been done on the antifungal potential of *Acacia* spp. [22] conducted an experiment to analyze the pharmacological activity of the ethyl acetate and ethanol extract of *Acacia nilotica* and *Turnera subulata* for its antifungal activity against six different fungal isolates namely, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida glabrata*, *Candida albicans* and *Penicillium chrysogenum*. In the study of [23], ethanol, acetone and hexane roots extracts of *Acacia catechu* was resistant to *Aspergillus niger*, *Fusarium oxysporum*, *Alternaria alternata*, *Rhizopus stolonifer* and *Macrophoma phaseolina*. [24] reported that *Acacia nilotica* bark and leaf extract showed antifungal activity with 12mm ZOI against *Aspergillus flavus*. [25] tested the ethanol extract of heartwood of *Acacia catechu* for antimycotic potential against *Aspergillus niger*, *Aspergillus fumigatus*, *Candida albicans*, *Mucor* spp. and *Penicillium marneffe*. In the study of [26], *Acacia nilotica* and *Acacia catechu* were most promising species. Both exhibited strong activity against two fungal species (*Aspergillus niger* and *Candida albicans*).

Table 1. Antifungal activity of ethanol and methanol extracts of stem-bark of *Acacia ampliceps* Maslin

S/No.	Fungal strains	Concentration of the extracts (µg/mL)					
		Ethanol		Methanol		Standard control	
		1000µg/mL	500 µg/mL	1000µg/mL	500 µg/mL	Nystatin (+ive) control)	DMSO (-ive)
		Zone of inhibition (mm)					
1	<i>Acremonium</i> spp.	16	12	20	16	22	-
2	<i>Aspergillus niger</i>	16	14	16	14	18	-
3	<i>Rhizopus</i> spp.	16	14	20	14	20	-
4	<i>Trichoderma</i>	18	14	22	16	23	-

1. *Acromonium* spp. (ethanol)

2. *Acromonium* spp. (methanol)

3. *Aspergillus niger* (ethanol)

4. *Aspergillus niger* (methanol)

5. *Rhizopus* spp. (ethanol)

6. *Rhizopus* spp. (methanol)

7. *Trichoderma* spp. (ethanol)

8. *Trichoderma* spp. (methanol)

Figure 1. Antifungal activity of ethanol and methanol extracted samples of *Acacia ampliceps* against fungal strains

Figure 2. Zone of inhibition of ethanol and methanol extracts of *Acacia ampliceps* stem bark as compared to standard antibiotic (Nystatin) against four fungal strains

Abd-Ulgadir *et al.* [27] observed the antimicrobial effect of aqueous and methanolic extract of stem and leaves of *Acacia nilotica* on five species of bacteria i.e. *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 53657), *Proteus vulgaris* (ATCC 6380), *Staphylococcus aureus* (ATCC 25933) and *Pseudomonas aeruginosa* (ATCC 27853) and one fungi species *Candida albicans* (ATCC 7596) by agar well diffusion method. Both methanolic and aqueous extracts showed strong antimicrobial activity [28] tested the aqueous extract of 52 plants from various families for their antifungal activity against eight significant species of *Aspergillus* such as *Aspergillus ochraceus*, *Aspergillus columnaris*, *Aspergillus fumigatus*, *Aspergillus flavipes*, *Aspergillus candidus*, *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus tamarii*. Among these tested plants, aqueous extract of *Acacia nilotica* showed noteworthy antifungal activity against the *Aspergillus* species. Antifungal activity of the water extract of the flower of *Acacia Saligna* (Labill.) was evaluated by [29] in micro-dilution method against three

mold fungi; *Fusarium culmorum* MH352452, *Rhizoctonia solani* MH352450, and *Penicillium chrysogenum* MH352451 that cause root rot, cankers, and green fruit rot, respectively, and it was concluded that by increasing the extract concentration, the percentage inhibition of fungal mycelial was significantly improved as compared to the control treatment, particularly against *P. chrysogenum*, suggesting that the use of *A. saligna* flower extract as a wood bio-preservative inhibited the growth of molds that cause discoloration of wood and wood merchandises.

Conclusion

Two different extracts i.e. ethanol and methanol of the stem-bark of *Acacia ampliceps* were used against four fungal strains. It was concluded that methanol extract was comparatively inhibitory to all fungal strains than ethanol extract.

Authors' contributions

Conceived and designed the experiments: MS Khan, Performed the experiments: Fatima, Analysed the data: MA Sajad, Contributed materials/ analysis/ tools: S Rahman, Wrote the paper: M Anwar.

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