

Antifungal activity of *Tamarixa phylla*(L.)Karst. stem-bark extract against some pathogenic fungi

Sadafbibi*, Muhammad Afzal, Kulsoombibi, Naziaaziz, Shahanaaziz and Abdur Raheem

Department of Botany, Hazara University, Mansehra

Corresponding author*

Sadafbibi,

Department of Botany,

Hazara University, Mansehra.

E-mail: guria209@yahoo.com

Abstract

The largest identified *Tamarixa phylla* (L.) Karst. belonging to family Tamaricaceae is traditionally an important plant used to cure various ailments. Three concentrations of crude ethanolic extracts 2000 ppm, 1000 ppm and 500 ppm were tested against six pathogenic fungi: *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium notatum* and *Saccharo myces cerevisiae* using five different solvents: acetone, chloroform, distilled water (DW), ethanol and methanol. Percent inhibition in growth of fungi was found to be dose dependent. The standard antifungal synthetic drug, Terbinafine, was used in different concentrations mixed with distilled water against different test fungi. Terbinafine completely controlled the growth of *A. flavus*, *A. fumigatus*, *A. niger*, *F. oxysporum*, *P. notatum* and *S. cerevisiae* with the concentrations of 65±0.58, 72±1.00, 70±1.15, 59±1.00, 60±0.58 and 80±0.58 (µg/ml of PDA medium), respectively. Chloroform was considered to be the most effective solvent preventing 97.68±0.58% growth of *F. oxysporum*, 9.37±0.33% in *A. niger*, 92.68±3.33% in *S. cerevisiae*, 91.46±2.08% in *A. fumigatus*, 88.48±0.88% in *A. flavus* and 87.95±1.15% in *P. notatum*. Statistically, the results were compared with negative control and most of the results were found to be highly significant (p≤0.000). Overall, our results suggest that *T. aphylla* stem-bark extract illustrated maximum percent inhibition with chloroform followed by ethanol, acetone, methanol and distilled water.

Keywords: Ethanol extract, inhibition, non-mycelial, incubated.

1. Introduction

Salt cedars (*Tamarix*spp.) belonging to family Tamaricaceae are invasive, exotic, deciduous, small shrubs or trees, native in the ancient world[1]. *Tamarixa phylla*(L.) Karst.is the largest identified species of the genus *Tamarix*, which is found in Central Asia, North Africa and Southeastern Europe[2]. The largest identified, *T. aphylla* is an evergreen tree that grows up to 18 m height[3]. *T. aphylla* leaves are used as an anti-inflammatory agent and in healing the wounds[4]. Leaves show considerable antioxidant activity[5]. *T. aphylla* extracts contain biologically active compounds, which work as substitute as the drug remedy used in the treatment of leishmaniasis[6]. *T. aphylla* leaves and young branches are given internally as decoction and externally as lotion[7]. Fumigation of the leaves has germicidal consequence. Bark is used as a bandage on wounds. *T. aphylla* bark and galls are used as astringent, aphrodisiac, tonic and are used for curing hepatitis, eczema and other skin diseases[8]. Decoction of the young twigs and leaves are applied for cure of swollen spleen. When the same decoction is supplemented with ginger, it is used for curing uterus infection[9]. The first identified glycosylated isoferulic acid, along with the new phenolics, tamarixetin and dehydrodigallic acid have been categorized from a crude extract of *T. aphylla*[10]. The primary phytochemicals investigation of aqueous extract of *T. aphylla* exhibited the occurrence of alkaloids, glycosides, saponins, tannins and phenolic compounds[11]. However, the present research work was conducted to evaluate the crude ethanolic extracts of *T. aphylla* stem-bark against some pathogenic fungi.

2. Material and Methods

Tamarixaphylla stem-bark was collected from Upper Chanai, opposite Commerce College, Mansehra.The plant part was identified by the Taxonomist, Dr. Jan Alam, Incharge, HazaraUniversity Herbarium, MansehraThe specimen of *T. aphylla*(Voucher No. 3733) was submitted to Hazara University, Herbarium..

2.1. Preparation of Extracts

The plant part was washed thoroughly and shade dried for two weeks and converted to fine powder by using electric grinder. Hundred (100) gram of the plant powder was dissolved in 500 ml of ethanol (Merck) to get crude ethanolic extract of *T. Aphylla* stem-bark. The extract was kept at room temperature for 48 hours, filtered and then kept open to evaporate the ethanol. After full evaporation, the residue obtained (1.45 gram). The extract was then stored in refrigerator at 4°C for further experiments.

2.2. Test Organisms

The fungal test organisms in the present study (*Aspergillus flavus* (Link), *Aspergillus fumigatus* (Fres), *Aspergillus niger* (Van Tiegh), *Fusarium oxysporum* (W.C. Snyder & H.N. Hansen), *Penicillium notatum* (Westling) and *Saccharomyces cerevisiae* (Meyen ex Hansen) were collected from Deptt. of Biotechnology, Kohat University of Science and Technology, Kohat (KUST).

2.3. Solvents Used

The solvents used in this technique were: acetone, ethanol, methanol, chloroform and distilled water (DW).

2.4. Antifungal Activity

Food poisoned technique [12] was used to determine the growth inhibition of fungi by the plant extract. Potato dextrose agar (PDA) was used as a medium for fungal culturing. The composition of the 1000 ml medium was agar 20g, sucrose 20g, potato peeled 200g and 1000ml distilled water. The test extracts of desired concentrations (500 ppm, 1000 ppm, 2000 ppm) in different solvents were poured into semi-solidified PDA medium test tubes. Then the test tubes were permitted to solidify in slanting position at room temperature. Each test tube was inoculated with an inoculum of 5 mm diameter, detached from the seven days old fungal culture.

For non-mycelial fungal growth, agar surface streak was applied. The test tubes were then incubated at $28 \pm 2^\circ\text{C}$ for seven days and were observed regularly. All experimental test tubes were in replicates of three in entirely randomized block plan [13]. 0.1 ml of every solvent was poured in experimental test tube and 5 mm diameter of inoculum from fungi was employed. Fungal growth was taken in millimeter (mm). After seven days growth of the control test tube, size of fungal colony was recorded in mm and inhibition in growth was noted along with the standard error. Terbinafine, was used as a standard antifungal drug.

Inhibition in the growth of every fungus due to different extracts in five different solvents was computed according to the given formula: $(C-T/C) \times 100$

Where, C is the linear growth noted from control tube (mm), T is the linear growth observed in experimental test tubes. Finally the calculated data was evaluated statistically.

3. Results

The synthetic drug, Terbinafine, was used as positive control during the antifungal assay of *T. aphylla* stem-bark. The inhibition in the linear growth was hundred percent. The doses of Terbinafine ($\mu\text{g/ml}$ of PDA medium), used against test fungi were: 65.00 ± 0.58 (*A. flavus*), 72.00 ± 1.00 (*A. fumigatus*), 70.00 ± 1.15 (*A. niger*), 59.00 ± 1.00 (*F. oxysporum*), 60.00 ± 0.58 (*P. notatum*) and 80.00 ± 0.58 (*S. cerevisiae*).

Antifungal assay of *T. aphylla* stem-bark extract was carried out in five solvents viz., methanol, ethanol, chloroform, DW, acetone and a wide range of results were observed [Table 1]. The extract was verified at three different doses, 500, 1000 and 2000 ppm. In methanol at lower concentration (500 ppm), inhibition in the growth of *A. niger* was $74.43\% \pm 2.84$, followed by *A. fumigatus* $55.33\% \pm 1.76$, and *S. cerevisiae* $11.24\% \pm 5.92$. With increase in dose concentration (1000 ppm), almost all the fungi exhibited relatively more growth than at 500 ppm.

Table 1: Antifungal Assay of *Tamarixa phylla* Stem-bark Extract in Five Different Solvents (Mean \pm SE)

| Test Organisms | Concentrations | Percent Inhibition in | | | | | Positive Control Dose (μ g/ml of PDA medium) |
|-------------------------------------|----------------|-----------------------|------------------|------------------|------------------|------------------|---|
| | | Methanol | Ethanol | Chloroform | DW | Acetone | |
| <i>A.flavus</i> | 500 ppm | 11.70 \pm 1.53 | 13.78 \pm 1.73 | 41.98 \pm 1.15 | 15.00 \pm 1.20 | 13.96 \pm 3.28 | 65.00 \pm 0.58 |
| | 1000 ppm | 41.13 \pm 2.08 | 45.28 \pm 2.33 | 72.43 \pm 5.21 | 38.21 \pm 1.76 | 38.74 \pm 1.20 | |
| | 2000 ppm | 62.90 \pm 4.63 | 68.90 \pm 2.33 | 88.48 \pm 0.88 | 64.29 \pm 2.85 | 67.57 \pm 1.53 | |
| Linear fungal growth in –ve Control | | 82.67 \pm 4.84 | 84.67 \pm 1.85 | 81.00 \pm 5.57 | 93.33 \pm 0.67 | 74.00 \pm 3.05 | |
| <i>A.fumigatus</i> | 500 ppm | 55.33 \pm 1.76 | 57.66 \pm 1.33 | 71.13 \pm 1.20 | 50.51 \pm 1.76 | 58.41 \pm 1.15 | 72.00 \pm 1.00 |
| | 1000 ppm | 73.12 \pm 1.76 | 67.51 \pm 1.85 | 82.93 \pm 1.53 | 69.55 \pm 1.76 | 70.30 \pm 1.53 | |
| | 2000 ppm | 86.16 \pm 1.20 | 80.65 \pm 1.85 | 91.46 \pm 2.08 | 79.93 \pm 0.88 | 81.69 \pm 1.85 | |
| Linear fungal growth in –ve Control | | 84.33 \pm 6.23 | 91.33 \pm 3.18 | 82.00 \pm 6.81 | 96.33 \pm 0.88 | 67.33 \pm 5.33 | |
| <i>A.niger</i> | 500 ppm | 74.43 \pm 2.84 | 79.70 \pm 6.00 | 74.43 \pm 2.00 | 60.55 \pm 4.36 | 84.46 \pm 0.33 | 70.00 \pm 1.15 |
| | 1000 ppm | 86.64 \pm 1.67 | 89.85 \pm 1.15 | 86.64 \pm 0.67 | 76.82 \pm 5.36 | 93.70 \pm 1.53 | |
| | 2000 ppm | 94.27 \pm 0.57 | 97.37 \pm 0.33 | 94.27 \pm 0.67 | 87.54 \pm 1.53 | 96.22 \pm 1.00 | |
| Linear fungal growth in –ve Control | | 87.33 \pm 1.45 | 88.67 \pm 0.88 | 87.33 \pm 1.20 | 96.33 \pm 1.20 | 79.33 \pm 4.63 | |
| <i>F.oxysporum</i> | 500 ppm | 31.08 \pm 4.62 | 54.10 \pm 5.90 | 69.50 \pm 2.33 | 3.94 \pm 2.03 | 68.94 \pm 3.84 | 59.00 \pm 1.00 |
| | 1000 ppm | 68.46 \pm 1.85 | 65.57 \pm 3.51 | 91.89 \pm 1.15 | 47.24 \pm 2.85 | 82.98 \pm 1.45 | |
| | 2000 ppm | 88.28 \pm 2.85 | 79.10 \pm 2.89 | 97.68 \pm 0.58 | 56.30 \pm 3.05 | 94.89 \pm 1.53 | |
| Linear fungal growth in –ve Control | | 74.00 \pm 6.66 | 81.33 \pm 5.78 | 86.33 \pm 2.67 | 84.67 \pm 1.45 | 78.33 \pm 8.74 | |
| <i>P.notatum</i> | 500 ppm | 49.44 \pm 2.33 | 45.66 \pm 4.36 | 64.94 \pm 6.01 | 51.48 \pm 3.18 | 65.87 \pm 1.67 | 60.00 \pm 0.58 |
| | 1000 ppm | 70.72 \pm 2.85 | 70.19 \pm 0.67 | 77.86 \pm 1.15 | 71.48 \pm 1.20 | 77.11 \pm 2.08 | |
| | 2000 ppm | 83.65 \pm 2.33 | 82.26 \pm 1.85 | 87.46 \pm 0.67 | 78.14 \pm 1.20 | 87.95 \pm 1.15 | |
| Linear fungal growth in –ve Control | | 87.67 \pm 1.45 | 88.33 \pm 4.05 | 90.33 \pm 2.33 | 90.00 \pm 2.89 | 83.00 \pm 4.16 | |
| <i>S.cerevisiae</i> | 500 ppm | 11.24 \pm 5.92 | 25.09 \pm 6.70 | 4.23 \pm 2.90 | 25.37 \pm 2.85 | 45.66 \pm 5.78 | 80.00 \pm 0.58 |
| | 1000 ppm | 64.65 \pm 5.61 | 77.22 \pm 3.53 | 67.80 \pm 2.60 | 42.16 \pm 9.73 | 84.47 \pm 4.10 | |
| | 2000 ppm | 80.72 \pm 2.64 | 88.80 \pm 1.45 | 85.17 \pm 2.18 | 86.57 \pm 2.52 | 92.68 \pm 3.33 | |
| Linear fungal growth in –ve Control | | 83.00 \pm 3.05 | 86.33 \pm 1.85 | 78.67 \pm 5.81 | 89.33 \pm 2.60 | 73.00 \pm 8.00 | |

However, at higher dose (2000 ppm), maximum percent inhibition recorded were; *A. niger* (94.27 \pm 0.57), *F. oxysporum* (88.28 \pm 2.85), *A. fumigatus* (86.16 \pm 1.20), *P. notatum* (83.65 \pm 2.33), *S. cerevisiae* (80.72 \pm 2.64) and *A. flavus* (62.90 \pm 4.63). At lower dose (500 ppm) in ethanol, percent inhibition recorded in the growth of *A. niger* was 79.70 \pm 6.00, followed by *A. fumigatus* 57.66 \pm 1.33, *F. oxysporum* 54.10 \pm 5.90, *P. notatum* 45.66 \pm 4.36, *S. cerevisiae* 25.09 \pm 6.70 and *A. flavus* 13.78 \pm 1.73. However, at the higher dose (2000 ppm), percent inhibition recorded were, 97.37 \pm 0.33 in *A. niger*, 88.80 \pm 1.45 in *S. cerevisiae*, 82.26 \pm 1.85 in *P. notatum* and 80.65 \pm 1.85 in *A. fumigatus*.

In chloroform at 500 ppm, inhibition in the fungal growth was found as; *A. niger* 74.43 \pm 2.00, *A. flavus* 41.98 \pm 1.15 and *S. Cerevisiae* 4.23 \pm 2.90. However, at the higher dose (2000 ppm), percent inhibition recorded in *F. oxysporum* was 97.68 \pm 0.58, followed by *A. Niger* 94.27 \pm 0.67, *A. fumigatus* 91.46 \pm 2.08, *A. flavus* 88.48 \pm 0.88, *P. notatum* 87.46 \pm 0.67 and *S. cerevisiae* 85.17 \pm 2.18. At 500 ppm, inhibition in the fungal growth was 25.37 \pm 2.85 in *S. cerevisiae*, 15.00 \pm 1.20 in *A. flavus* and 3.94 \pm 2.03 in *F. Oxysporum* in DW. At 2000 ppm, the percent inhibition in the growth of *A. niger* was 87.54 \pm 1.53, followed by *S. cerevisiae* (86.57 \pm 2.52), *A. fumigatus* (79.93 \pm 0.88), *P. notatum* (78.14 \pm 1.20), *A. flavus* (64.29 \pm 2.85), *F. oxysporum* (56.30 \pm 3.05).

By analyzing the antifungal assay of *T. aphylla* stem-bark extract in acetone, percent inhibition observed at 500 ppm was 58.41 \pm 1.15 in *A. fumigatus*, 45.66 \pm 5.78 in *S. cerevisiae* and 13.96 \pm 3.28 in *A. flavus*. At 2000 ppm, the inhibition was enhanced to 96.22 \pm 1.00 in *A. niger*, followed by 94.89 \pm 1.53 in *F. oxysporum*, 92.68 \pm 3.33 in *S. cerevisiae*, 87.95 \pm 1.15 in *P. notatum*, 81.69 \pm 1.85 in *A. fumigatus* and 67.57 \pm 1.53 in *A. flavus*. After evaluating the antifungal screening of *T. aphylla* stem-bark extract in five solvents, the data was analyzed by one way analysis of variance (ANOVA) most of the cases were found as highly significant ($p \leq 0.000$).

4. Conclusion and Discussion

The present study revealed that the plants are important source of potentially useful substances for the development of new antifungal agents. In this study, the antifungal activity of *T. aphylla* stem-bark extract was carried out by food poisoned method. All the investigated plant extracts tested in different solvents exhibited different degree of

antifungal activity in dose dependent manner. The antifungal assay of *T. aphylla* stem-bark show that chloroform extract has highest antifungal activity (97.68%±0.58) against growth of *F. oxysporum* followed by ethanolic extract (97.37%±0.33) against *A. niger*, suggesting that the active compounds of *T. aphylla* stem-bark extract is better soluble in chloroform than other solvents. There are many reports available on the antimicrobial activities of plant products[14]-[20].

Our results regarding the antifungal potential of the ethanol extract of *T. aphylla* are in harmony with [21] who displayed significant antimicrobial effects of *T. aphylla* stem and leaves. [5] also mentioned that the crude ethanolic extract of *T. aphylla* leaves exhibited significant antifungal activity of *T. aphylla* against *A. flavus* and *A. fumigatus*. [22] examined the leaves methanolic extracts from *T. aphylla* for their antiaflatoxigenic and antifungal actions against *A. flavus*, which displayed almost similar results to the present studies.

Similarly, the results of this study regarding the ethanol extract of *T. aphylla* are in agreement with the findings of other authors [5][16][21]-[24]. Finally the results of the current study revealed good antifungal activity of *Tamarix aphylla* against six fungal species i.e *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *F. oxysporum*, *P. notatum* and *S. cerevisiae*.

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