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ANTIFUNGAL AND ANTIOXIDANT ACTIVITY OF TAMARIX APHYLLA L. LEAF EXTRACT

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ABSTRACT: The largest identified *Tamarix aphylla* (L.)Karst belonging to family Tamaricaceae was extracted with acetone and ethanol ,used to evaluate total phenolics content. The antioxidant activities of these extracts were determined by 2, 2'- Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. Total phenolics content were high in ethanol extract (993.11 ± 22.50 mg GAE/g) as compared to acetone extracts. DPPH free radical scavenging activity of the *Tamarix aphylla* extracts varied widely and it increased with increase of concentration levels. Antioxidant activity of ethanol extract was found to be maximum (81.23 ± 0.41) than acetone extracts. Three concentrations of crude ethanolic extracts 2000 ppm, 1000 ppm and 500 ppm were tested against two pathogenic fungi: *Fusarium oxysporum, Penicillium notatum* using two different solvents. Terbinafine completely controlled the growth of *F.oxysporum* and, *P.notatum* with the concentrations of 59±1.00 and 60±0.58 (μg/ml of PDA medium), respectively. Overall, our results suggest that *T. aphylla* leaf extract illustrated maximum percent inhibition with acetone (94.89 ± 1.53) in *F.oxysporum* followed by (87.95 ± 1.15) in *P.notatum.*

Keywords: *Tamarix aphylla*, total phenolics, antioxidant assay, antifungal activity, inhibition

INTRODUCTION

Medicinal plants have been playing a pivotal role in improving human life. The medicinal value of these plants is related in their phytochemical components which produce definite physiological actions on human body. The most important of these components are alkaloids, tannins, flavonoids and phenolic compounds. Several trees have been reported to exhibit antioxidant activity and a great potential source of antioxidant are polyphenols (Bocco *et al*., 1998). Solvent and process variables must be carefully chosen to optimize their extraction. The extraction yield and antioxidant activity of the extracts highly depend on solvent polarity which determines both quantitatively and qualitatively the extracted antioxidant compounds. *T. aphylla* leaves are used as an anti-inflammatory agent and in healing the wounds (Abdallah and Ghazali 2013). Leaves show considerable antioxidant activity (Zain *et al.*, 2012). *T. aphylla* extracts contain biologically active compounds, which work as substitute as the drug remedy used in the treatment of leishmaniasis. (Iqbal *et al*., 2013) 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 (Marwat *et al*., 2009). Little literature data was available on the antioxidant activity of different solvent extracts of leaves of *T. aphylla*. So the present study was conducted to estimate total phenols in different extracts and their antioxidant, antifungal activity of these extracts.

MATERIALS AND METHODS

The leaves of *T. aphylla* were collected from CCS, Haryana Agricultural University campus, Hisar.

Chemicals: The commercially available chemicals from Sigma Aldrich, Qualigens, Merk and Ranbaxy, of high purity, were used for various experimental procedures.

Extraction of plant materials : 100 gm samples of plant material were extracted separately with ethanol by refluxing for 6 hours and the process repeated three times. The solvent was removed to get extractives. These extracts were filtered and concentrated under reduced pressure and used for estimation of following parameters:

1. Total Phenols
2. Antioxidant activity by 2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) method

Preparation of extracts: The acetone and ethanol extracts were prepared under reflux conditions for 6 hours and process repeated three times.The solvent of the combined filtrates was evaporated using a rotatory evaporator.

Determination of total phenolics content

The total phenolics were determined by Folin-Ciocalteu regent method (Singleton and Rossi, 1965) using gallic acid as a standard. The absorbance was measured at 730nm using Spectronic 20 (Milton Roy Company) spectrophotometer against a blank. The results were expressed as the equivalent to milligrams of gallic acid per gram of extract (mg GAE/g).

Determination of antioxidant activity

2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method

The antioxidant activity of the extracts was evaluated by DPPH free radical scavenging method of Hatano *et al*., (1988). The absorbance of the sample was measured

at 517nm using the spectrophotometer Spectronic 20 (Milton Roy Company). The antioxidant activity was expressed as (%DPPH x SC) and calculated using:

$$\%DPPH \times SC = \{(A_{cont}-A_{samp})/A_{cont}\} \times 100$$

Here, A_{cont} is the absorbance of control and A_{samp} is the absorbance of sample.

Determination of Antifungal activity method

Food poisoned technique 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±2°C for seven days and were observed regularly. All experimental test tubes were in replicates of three in entirely randomized block plan (Afzal *et al*., 2005). 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 three different solvents was computed according to the given formula: (C-T/C) x100 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.

RESULTS

Total phenolics content

Phenols are aromatic secondary plant metabolites which are the most widely spread in nature. This diverse group of phenolic compounds include polyphenols,

phenolic acids, anthocyanins, etc. act as potential natural antioxidants due to their ability to scavenge free radicals. It is well known that the antioxidant activity of phenols is mainly due to their redox properties, H-donors and singlet oxygen quenchers. Therefore, the amounts of total phenols in the extracts were determined. Our results showed that the content of total phenols varied from 993.11 ± 22.50 mg GAE/g (ethanol extract) and 772.32 ± 1.16 mg GE/g (acetone extract) in *T. aphylla* leaves.

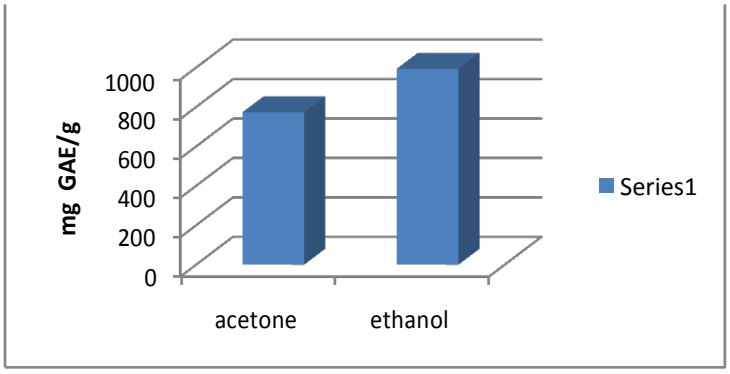

Fig. 1: Total phenolics contents of *T. aphylla.* Leaf extract.

Antioxidant activity by DPPH method

Antioxidants can exercise their protective effect by scavenging free radicals, which are the main propagators of oxidation of lipids in various biological systems. Lipid oxidation is a complex process which can be influenced by various factors like temperature, light and presence of initiators (metal enzymes). So, different methods are needed for monitoring oxidation processes to assess primary and secondary oxidation changes and the efficiency of antioxidants. So, the present study is aimed at the evaluation of the antioxidant activity of different extracts of leaves of *T. aphylla.*

DPPH (2,2'-diphenyl-1-picrylhydrazyl) is a stable and deep violet colour organic nitrogen radical, which is used in the evaluation of radical scavenging activity(Yon Gadow *et al.*, 1997). Alcoholic solutions of $DPPH^{\bullet}$ have a characteristic absorption maximum at 517nm. The essence of this methods is that when an electron or hydrogen atom donating antioxidant (AH) is added to $DPPH^{\bullet}$, it reacts with DPPH to form 1,1-diphenyl-2-picrylhydrzine, which is a non-radical colourless form and does not absorb at

517nm. The degree of discoloration indicates the free radical scavenging activity of the samples.

$$DPPH^{\bullet} + AH \rightarrow DPPH\text{-}H + A^{\bullet}$$

All the extracts were screened for radical scavenging activity against DPPH.Free radical scavenging activity (%) of the acetone and ethanol extracts of *T. aphylla.* leaf extract varied widely and it increased with increase of concentration levels . DPPH free radical scavenging activity (%) of *T. aphylla.* ranged from 12.11 to 81.23% (ethanol extract) and from 9.1 to 72.5 % (acetone extract) at different concentration levels ranging from 0.25 to 5.0 mg/mL (Table 1). The IC_{50} value of ethanol extract was lowest i.e. 0.077 mg/mL followed by 0.110 mg/mL of acetone extract ,thereby showing that ethanol extract has highest activity followed by acetone extracts. This might be due to the presence of phenols which are generally more soluble in ethanol. The highly polar solvent extracts were the most active in the $DPPH^{\bullet}$ test system.

Table 1:DPPH free radical scavenging activity (%) of different *T. aphylla.* Leaf extract.

Extracts / Conc. (mg/mL)	DPPH Free Radical Scavenging Activity (%)						IC_{50} (mg/mL)
	0.4	0.2	0.1	0.05	0.02	0.01	
Acetone	72.5±0.38	69.5±0.11	52.9±0.25	23.5±0.26	11.5±0.31	9.1±0.36	0.110
Ethanol	81.23±0.41	76.45±0.02	63.12±0.21	48.34±0.32	24.34±0.21	12.11±0.31	0.077

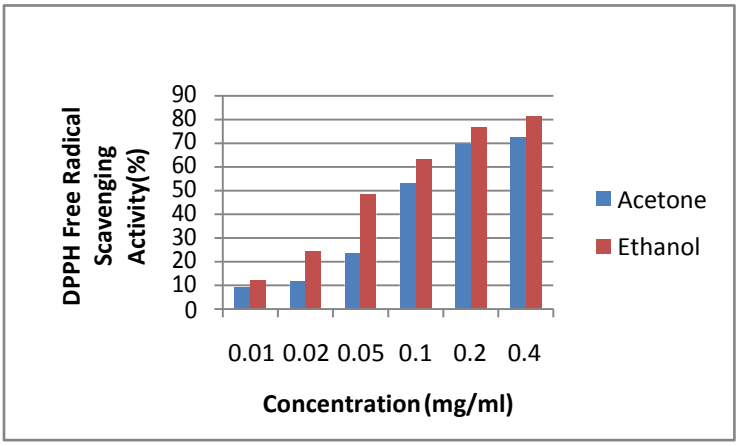

Fig.2 DPPH free radical scavenging activity (%) of different *T. aphylla.* Leaf extract.

Antifungal activity

The synthetic drug, Terbinafine, was used as positive control during the antifungal assay of *T. aphylla* leaves. The inhibition in the linear growth was hundred percent. The doses of Terbinafine (µg/ml of PDA medium), used against test fungi were: 59.00±1.00 (*F.oxysporum*),60.00± 0.58 (*P.notatum*).

Antifungal assay of *T. aphylla* leaf extract was carried out in two solvents viz., acetone, ethanol and a wide range of results were observed [Table 2].The extract was verified at three different doses, 500, 1000 and 2000 ppm. At lower dose (500 ppm) extract in acetone, percent inhibition observed at 500 ppm was 68.94%±3.84in *F.oxysporum*, and 65.87%±1.67 in *P.notatum*. At 2000 ppm, the inhibition was enhanced to 94.89%±1.53 in *F.oxysporum* and 87.95%±1.15 in *P.notatum.*

By analyzing the antifungal assay of *T. aphylla* leaf extract extract in ethanol, percent inhibition recorded in the growth of *F.oxysporum was* 54.10%±5.90 followed by *P.notatum* 45.66%±4.36. However, at the higher dose (2000 ppm), percent inhibition recorded was 82.26%±1.85 in *P.notatum* and (79.10%±2.89) in *F.oxysporum.*

After evaluating the antifungal screening of *T. aphylla* leaf extract extract in two solvents, the data was analyzed by one way analysis of variance (ANOVA) most of the cases were found as highly significant ($p \leq 0.000$).

Table 2: Antifungal Assay of *Tamarixa phyla* Leaves Extract in two Different Solvents (Mean ± SE)

Test Organisms	**Concentrations**	**Percent Inhibition in**		**Positive Control Dose (µg/ml of PDA medium)**
		Acetone	**Ethanol**	
F.oxysporum	500 ppm	68.94±3.84	54.10±5.90	59.00±1.00
	1000 ppm	82.98±1.45	65.57±3.51	
	2000 ppm	94.89±1.53	79.10±2.89	
Linear fungal growth in –ve Control		78.33±8.74	81.33±5.78	
Test Organisms	**Concentrations**	**Percent Inhibition in**		**Positive Control Dose (µg/ml of PDA medium)**
		Acetone	**Ethanol**	
P.notatum	500 ppm	65.87±1.67	45.66±4.36	60.00± 0.58
	1000 ppm	77.11±2.08	70.19±0.67	
	2000 ppm	87.95±1.15	82.26±1.85	
Linear fungal growth in –ve Control		88.00±4.16	88.33±4.05	

CONCLUSION AND DISCUSSION

Our results demonstrated that ethanol extract of leaves of *T.aphylla* has present strong and potent scavenging capacity against free radical DPPH and with this same solvent we recorded the higest content polyphenols compared to other solvents The antifungal assay extract of leaves of *T.aphylla* in acetone extracts shows that percent inhibition recorded was highest (94.89%±1.53) against growth of *F.oxysporum* followed by (87.95%±1.15) P.notatum and can be incorporated in pharmacological and medical use..

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