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Antioxidant activity total phenolic and flavonoid content of aerial parts of *Tamarix gallica*

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Abstract

The present study was designed to investigate the antioxidant activities ofmethanolic extract of aerial parts of Tamarix Gallica and to evaluate the phenolic and flavonoid content of the plant.

The antioxidant activity of methanolic extract was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging and ferric-reducing/antioxidant power (FRAP) assays. The total phenolic content was determined according to the Folin-Ciocalteu procedure and calculated in terms of gallic acid equivalents (GAE). The flavonoid content was determined by the gravimetric method in terms of quercetin equivalents. The methanolic extract of aerial parts of Tamarix Gallica showed high antioxidant activity as compared to standard ascorbic acid used in the study. The results were found as 6.99498mg/100g for total content of phenols, 47.61905mg/100g for total flavonoid content and $IC_{50}$of 0.5mg/ml for the antioxidant activity. Tamarix Gallica is a potential source of natural antioxidant for the functional foods and nutraceutical applications..

Keywords: Tamarix Gallica, Antioxidant, Total Phenols, Flavonoid, DPPH, ferric reducing assay.

Introduction

Antioxidant means "against oxidation." In the oxidation process when oxygen interacts with certain molecules, atoms or groups of atoms, odd (unpaired) number of electrons called free radicals can be formed. In a human body, these highly reactive radicals are known to cause oxidation damage or death of the cells by reacting with important cellular components such as DNA, or the cell membrane. Many diseases such as cancer, atherosclerosis, diabetes and liver cirrhosis are associated with free radical damage to body cells and large biomolecules such as lipids, DNA and proteins [1-3].

Antioxidants are the molecules which can safely interact with free radicals to terminate the chain reaction before vital molecules are damaged and are intimately involved in the prevention of cellular damage - the common pathway for cancer, aging, and a variety of diseases. The human body contains antioxidant enzymes like catalase, peroxidase and superoxide dismutase that provide defence against free radical damage. Increased consumption of whole grains, fruits and vegetables provides natural antioxidants such as vitamin C, tocopherol, carotenoids, polyphenolics and flavonoids to exert an antioxidant action [4].

Recently, there have been great efforts to find safe and potent natural antioxidants from various plant sources. Researchers have identified antioxidant activities in various plants and attribute it to the presence of flavones, isoflavones, flavonoids, anthocyanins, coumarins, lignans, catechins and isocatechins [5-7].The Phenolic compounds, secondary metabolites of plants are one of the most widely occurring groups of phytochemicals that exhibit antiallergenic, antimicrobial, anti-artherogenic, antithrombotic, anti-inflammatory, vasodilatory and cardioprotective effects [6-9]. Due to the presence of the conjugated ring structures and hydroxyl groups, many phenolic compounds have the potential to function as antioxidants by scavenging or stabilizing free radicals involved in oxidative processes through hydrogenation or complexing with oxidizing species that are much stronger than those of vitamins C and E [10].

In the recent years there has also been an increasing interest in the rate of free radical mediated damage in the aetiology of human diseases. Synthetic antioxidants such as butylatedhydroxyanisol (BHA) and butylated hydroxyl toluene (BHT) etc. have been found to be associated with the risk of cancer occurrence and liver damage in human [11,12].Therefore, the development and isolation of natural antioxidants from natural plant has become the focus of the research of antioxidant, and many studies have suggested that consumption of certain natural antioxidants lead to reduction in oxidative stress and the development of human major diseases [13,14].

Based on the traditional claims of Tamarix Gallica and the lack of scientific studies of its potential pharmacological properties, the objective of this study is to evaluate the antioxidant activity through direct free radical scavenging method and indirect ferric reducing assay and also to elucidate total phenolic content (TPC) and polyphenolic flavonoid content of the plant.

TamarixGallica belongs to the family Tamaricaceae and is deep-rooted, gregarious bushy ornamental shrubs or trees native to the Mediterranean region and northern China. About 125 species of the plant have been reported from different regions of Europe, USA, Asia and Africa. The plant grows in saline or water logged soil up to 17 to 19 feet. As it ages the smooth, reddish-brown bark of younger plants turns brownish-purple, ridged, and furrowed. Feathery, needle-like leaves, often encrusted with salt secretions, grow on its thin branches. Masses of small, pink flowers blossom on the ends of its branches from June to August

Classification	
Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Dilleniidae
Order	Violales
Family	Tamaricaceae – Tamarix family
Genus	Genus Tamarix L. – tamarisk

Materials and methods

Plant Material and Preparation of methanolic extract

The aerial parts of TamarixGallica for the study were procured from Nirankar Herbs; 2211/4344/Aggarwal market, Tilak Bazar, kharibawri market, Delhi-6 (India) locally in the month of February. The authentication of leaves was done by Dr. H.B. Singh, Head, Raw material, Herbarium and Museum division, National Institute of Science Communication and Information Resources (NISCAIR), PUSA New Delhi, India.

The air-dried aerial parts were made in to a coarse powder and macerated with methanol for 48 hrs. Extraction was done through Soxhlet apparatus. After extraction, the solvent was distilled off and the extract was concentrated under reduced pressure on a water bath at a temperature below 50°C to give a semisolid brown syrupy consistency residue, which was stored in a closed bottle and refrigerated at temperature below 4°C until tested.

Chemicals

Diphenyl-1-picryl hydrazyl (DPPH), Ascorbic acid, Folin–Ciocalteau reagent, Gallic acid and all other reagents and solvents of analytical and HPLC grade were purchased from Merck Co (Germany).

Preliminary phytochemical screening of extract

The methanolic extract was tested by Khandelwal method [15] for alkaloids, glycosides, reducing sugars, tannins, fixed oils and fats, proteins and free amino acids. The results are shown in Table 1.

Table 1 - Preliminary phytochemical screening of methanolic extract of TamarixGallica.

Extract	Steroid	Alkaloid	Tannin	Carbohydrate	Quercetin glycosides	Flavonoid	Saponin
TamarixGallica	-	+	+	-	+	+	+

(+) Methanolic extract ofTamarixGallicapresent; (-) Methanolic extract ofTamarixGallicaabsent

In-Vitro Antioxidant Activity

DPPH free radical scavenging activity of methanolic extract

The antioxidant activity of the plant extracts and the standard was assessed by Hinneburg method [16] on the basis of the radical scavenging effect of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH)-free radical activity. The diluted working solutions of the test extracts were prepared in methanol and ascorbic acid was used as standard in 0.06,0.12,0.25, 0.5 mg and 1 mg/ml solution.1 ml of 0.002% of DPPH solution prepared in methanol was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 516nm using UV-visible Spectrophotometer (Shimadzu, UVV-1700, Japan). Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below [17].

$$\% \text{ Inhibition} = \frac{[\text{Absorbance of control} - \text{Absorbance of test sample}]}{[\text{Absorbance of Control}]} \times 100$$

Each experiment was carried out in triplicate and results were expressed as mean % antiradical activity ± Standard Deviation.

Reducing power by $FeCl_3$

Reducing power of the extract was evaluated by Oyaizu method [18]. 2ml of different concentrations of TamarixGallica extract (100-3000 µg/ml) and standard solution were mixed with phosphate buffer(0.2 M, pH 6.6) and 1% Potassium ferricyanide solution. This mixture was incubated at 50° C for 20 min. After cooling 2.5 ml of 10% Trichloro acetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant was mixed with 2.5 ml of distilled water and 1 ml of 0.1% ferric chloride and kept for 10 min. The absorbance of resulting solution was measured at 700 nm. Increased absorbance of the reaction mixture was associated with increased reducing power of the extract. Ascorbic acid was used as the standard. Phosphate buffer(pH 6.6) was used as blank solution. Control was prepared in similar manner excluding samples.

Determination of the total phenolic content

Total phenolic compounds in samples were determined spectrophotometrically with Folin-Ciocalteu reagent using Gallic acid as standard according to Ayurvedic Pharmacopoeia of India (API, 2002) [19]. Sample was diluted with distilled water. One milliliter of Folin-Ciocalteu reagent was then added and the content in the flask was mixed thoroughly. After 3 min, 3 ml of 2g/100ml Sodium Carbonate solution was added and the mixture was allowed to stand for 2hr with intermittent shaking. The absorbance was measured at 760 nm using spectrophotometer. The total phenolic content was expressed based on Gallic acid equivalent (GAE).

Determination of the total flavonoid content

The flavonoid content was determined according to the gravimetric method [20]. 5 gram of the powdered sample was boiled in 50ml of 2MHCl solution for 30 minutes under reflux. The boiled mixture was allowed to cool and then filtered through Whatman No 42 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with drop. 10ml of ethyl acetate extract which contained flavonoid was recovered, while the aqueous layer was discarded. A pre weighed Whatman filter paper was used to filter second (ethyl-acetate layer), the residue was then placed in an oven to dry at 60°C. It was cooled in a dessicator and weighed. The quantity of flavonoid was determined using the formula.

$$\% \text{ Flavonoid} = \frac{W2-W1}{\text{Weight of Sample}} \times 100$$

Where -
W1= Weight of empty filter paper,
W2= Weight of paper + Flavonoid
The resulting weight difference gave the weight of flavonoid in the sample.

Results

The results of the study are shown in Table 2-3 and Fig 1-3

Table 2 – Antioxidant and % DPPHactivity of TamarixGallica as compared to Standard Ascorbic acid (DPPH method)

	Absorbance			% DPPH	
Concentration (mg/ml)	Standard (Ascorbic acid)	Sample (TamarixGallica)	Sample (Blank)	Standard (Ascorbic acid)	Sample (TamarixGallica)
0.00	0	0	0	0	0
0.06	0.402 ±0.001	1.098 ±0.001	1.487	72.9657	26.1600
0.12	0.340 ±0.004	1.078 ±0.002	1.487	77.1351	27.505
0.25	0.229 ±0.002	0.979 ±0.003	1.487	84.5998	34.1627
0.50	0.120 ±0.003	0.736 ±0.001	1.487	91.9300	50.5043
1.00	0.038 ±0.001	0.465 ±0.003	1.487	97.4445	68.7289

Table 3 - Total Phenolic and Flavonoid content in sample TamarixGallica.

Total Phenolic content	6.99498 mg/100gm
Total Flavonoid content	47.61905 mg/100gm

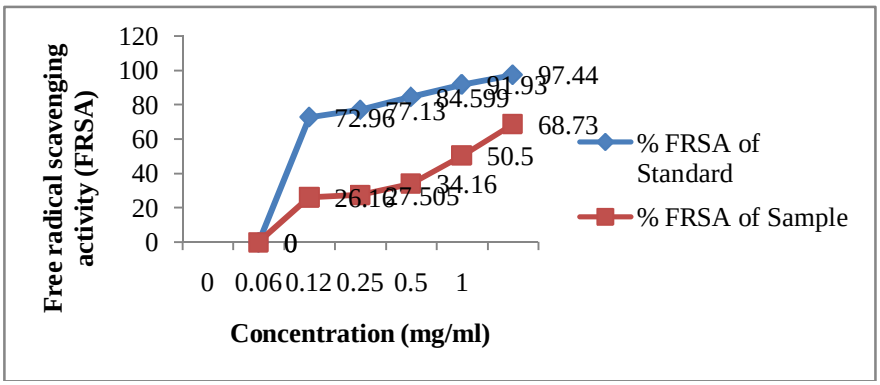

Fig 1 - Free radical scavenging activity (FRSA) of Sample TamarixGallica as compared to Standard Ascorbic acid.

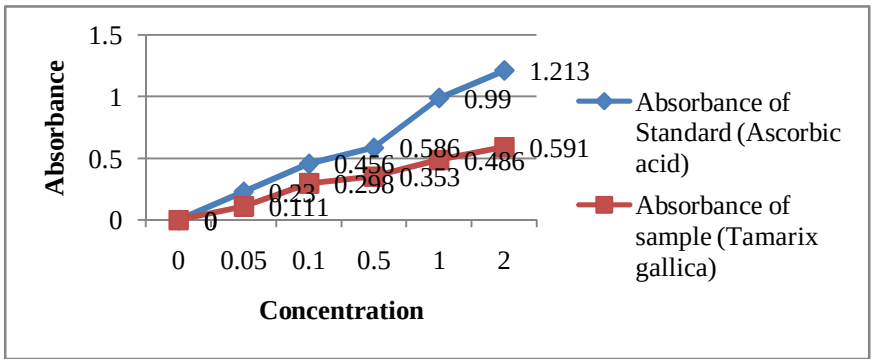

Fig 2 - Reducing power of Sample TamarixGallicaby $Fecl_3$as compared to Standard Ascorbic acid.

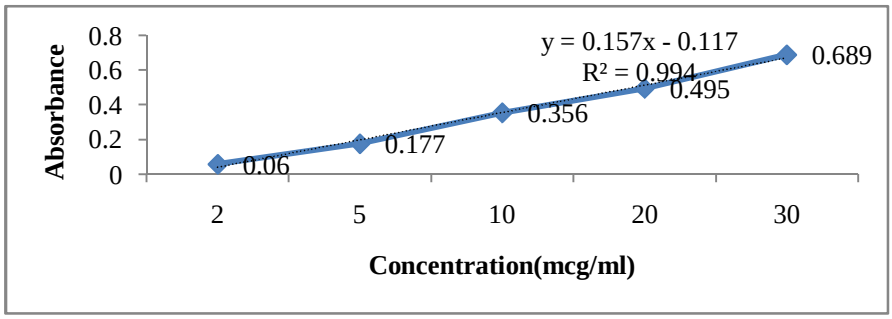

Fig 3 – Standard curve of Gallic acid with R^2 of 0.9994 to determine the total Phenolic content using the Folin-Ciocalteu reagent.

Statistical Analysis

Statistical analysis was performed based on one way Analysis of variance (ANOVA) at confidence level 95%. Linear regression to correlate total phenolics and flavonoid content with antioxidant activity was carried using Excel 2010.All data are presented as mean ± standard deviation for at least three replications for each prepared sample.

Discussion

The methanolic extract from the aerial parts of TamarixGallica was investigated for total phenolic content, flavonoid content and antioxidant activity.

The methanolic extract from aerial parts of TamarixGallica were tested at various concentrations for their antioxidant activity using indirect (DPPH radical scavenging assay) and direct (ferric reducing power assay) method.

The DPPH radical scavenging activity assesses the capacity of an extract to donate hydrogen or to scavenge free radicals. DPPH radical is a stable free radical which on reacting with an antioxidant compound (that can donate hydrogen) gets reduced to diphenylpicrylhydrazine (DPPHH). In the test conducted this process was evident by the switch in colour (that is, from deep-violet to light-yellow) measured spectrophotometrically. The methanolic extract of TamarixGallica decolorized the purple colour of DPPH to the yellow of DPPHH with an IC_{50} value of 0.5 mg/ml.IC_{50} value is the parameter which is used to measure the radical scavenging activity of extract and is defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals in this specified time period. The smaller IC_{50} value corresponds to the higher antioxidant activity of the plant extract [21]. The decrease in absorbance of DPPH solution (from purple to

yellow) is hence attributed to intrinsic antioxidant activity and the acceleration of reaction between DPPH and antioxidant as shown in Table 2. The radical scavenging activity (%) in the TamarixGallica samples was found to increase gradually in a dose dependent manner as shown in Fig 1.

Ferric Reducing Antioxidant Power (FRAP) assay is the only assay that directly measures antioxidants in a sample. The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric reaction. In the current study the reducing power of the extract was determined by direct electron donation in the reduction of ferri cyanide $[FeCN)6]^{3-}$ to ferro cyanide $[Fe(CN)_6]^{4-}$. The product was visualized by addition of free Fe^{3+} ions after the reduction reaction, by forming the Perl's Prussian blue colour complex, $(Fe^{3+})_4[Fe^{2+}(CN\text{-})_6]_3$, and quantified by absorbance measurement at 700 nm [22]. A dose dependent increase in absorbance and reducing power of TamarixGallica extract as shown in Fig2 is indicative of hydrogen donating capacity of the extract.

Phenolics or polyphenols are secondary plant metabolites that are ubiquitously present in plants and plant products. Many of the phenolics show high levels of antioxidant activities due to their redox properties[23].Generally the phenolic compounds are found to work by neutralizing lipid free radicals and preventing decomposition of hydro peroxides into free radicals [24]. Phenolic compounds are a class of antioxidant agents which act as free radical terminators[25]. In the current study, Total phenol content(TPC) of extract was determined by Folin-Ciocalteau (F-C) assay using Gallic acid as a standard phenolic compound. F-C assay for total phenolics contents being a fast and simple method was selected. The F-C method is based on oxidation of phenolics by molybdotungstate present in F-C reagent to yield a colored product with λmax 745 – 750 nm [26]. A linear calibration curve of Gallic acid, in the range of 2–30 µg/ml with coefficient of determination (R^2) value of 0.994, is shown in Fig 3. The total phenolic content (TPC) of the extract of TamarixGallicais demonstrated in Table3.

Flavonoids are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants, characterized by a benzo-γpyrone structure. Various studies have shown that flavonoids show antioxidant activity and their effects on human nutrition and health is considerable.The mechanism of action of flavonoids is through scavenging or chelating process [27, 28]. The total flavonoid content in the current study was expressed in milligram per gram dry material of extracts.The flavonoid content of the extract was measured by gravimetric method. The result of 47.61905 mg/100gm is shown in Table 3.

The preliminary phytochemical screening of extract indicated the presence of polyphenolic compounds such as flavonoids, phenolic acids, tannins and coumarins.Polyphenols particularly flavonoids and tannins are well known natural antioxidant [29].The antioxidant activity of the aerial parts of methanolicextract ofTamarixGallicais attributed to the presence of phenolic compounds.

Conclusion

The study supports the contention that traditional medicines remain a valuable source in the discovery of antioxidants. In the current study, aerial parts of TamarixGallica have shown potential as a good source of natural antioxidants. The phenolic compounds found in the plant are believed to be responsible for the antioxidant properties of the extract. TamarixGallica is a potential source of antioxidant for the functional foods and nutraceutical applications.

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