Original Research

In vitro comparative evaluation of *Tamarix gallica* extracts for antioxidant and antidiabetic activity

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Impact statement

Numerous studies have been conducted on oxidative stress, diabetes, and disorders that are connected to these diseases. Oxidative stress is the major cause of many chronic diseases and diabetes is a global health issue now a days. We have identified the antioxidant and antidiabetic properties of *Tamarix gallica* that are correlated with phenolic content (TPC and TFC) of the plant. Our data suggest that *Tamarix gallica* have the high phenolic content. The results further demonstrate that *Tamarix gallica* have property to overcome the oxidative stress and have the ability to manage diabetes by inhibiting α-amylase and α-glucosidase activities. High phenolic content of the plant play important role in the antioxidant and antidiabetic property. Even more, *Tamarix gallica* antioxidant property also a key factor of antidiabetic property of the plant.

Abstract

Tamarix gallica known as Jhau is traditionally used as expectorant, liver tonic, laxative, astringent, and antidiarrheal. The current study was proposed to determine the *in vitro* antioxidant, antidiabetic properties of the methanolic, ethanolic, and aqueous extracts of *Tamarix gallica* arial part, subsequently the phytochemical evaluation. Hence, *Tamarix gallica* arial part extracts were extracted with methanol (MthTg), ethanol (EthTg), and distilled water (AqTg). Extracts phytochemical analysis were accomplished to identify the phenolic components (TPC and TFC). Extracts antioxidant property was evaluated by DPPH, FRAP, and ABTS assay. For antidiabetic property, α-amylase and α-glucosidase inhibitory activities were assessed. One Way ANOVA was applied statistically by means of software SPSS Statistics 23 and attained data were definite as mean $\pm$ standard deviation. Result revealed that EthTg have the maximum TPC and TFC levels than MthTg and AqTg. Antioxidant property in relations of DPPH (lowest $IC_{50} = 1.309 \pm 0.31$), FRAP (323.51 ± 2.32), and ABTS (266.97 ± 25.14) assay was also highest in EthTg. EthTg was also exposed highest α-amylase and α-glucosidase inhibition activity with lower IC_{50} (1.116 ± 0.051; 0.402 ± 0.2, respectively). The extracts antioxidant and antidiabetic activities order was as EthTg > MthTg > AqTg. TFC and TPC also revealed directly proportional correlation with antioxidant, and antidiabetic properties of the *Tamarix gallica* arial part extracts. Results noticeably stated that the ethanolic extract of *Tamarix gallica* have the highest antioxidant and antidiabetic properties. *Tamarix gallica* has competency to reduce the oxidative stress and can be utilized in the management of diabetes.

Keywords: *Tamarix gallica*, phytochemicals, antioxidant, antidiabetic

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Introduction

Antioxidant defenses nullify the detrimental properties of reactive oxygen and nitrogen species (RONS) that are produced by a number of exogenous and endogenous processes. The disproportion among these reactive species synthesis and antioxidant defenses causes oxidative stress.[1] NADPH oxidase, angiotensin II, lipoxygenase, and myeloperoxidase are all endogenous sources of these reactive species.[2] Alcohol, tobacco, water and air pollution, heavy or transition metals, industrial solvents, drugs (e.g. bleomycin, cyclosporine, gentamycin, and tacrolimus), cooking (e.g. waste oil and smoked meat), and radiation are all exogenous sources of RONS, which are metabolized into free radicals inside the body.[3] Several disorders e.g. cancer, cardiovascular diseases, renal disease, fatty liver, diabetes, chronic obstructive pulmonary disease, and neurological diseases are linked to oxidative stress.[4–9] A serious global public health problem that affects people all across the world is diabetes mellitus.[10] Diabetes mellitus is a metabolic disorder that generates rise in free radical ion production and a reduction in antioxidant capability that leads to macro- and microvascular consequences.[11] Recent data suggest that a redox imbalance causes oxidative stress, which contributes to the onset and

progression of diabetes and its consequences, via modulating signaling pathways involved in cell dysfunction and insulin resistance. Reactive oxygen species (ROS) can also directly oxidize proteins implicated in the diabetic process (a process known as redox modification).[10]

A multitude of processes, including oxidative stress, contribute to diabetes-related cardiovascular disease. Consequently, it's necessary to keep the body's oxidative stress and level of sugar in check. Nature has always provided a plentiful supply of key substances linked to valued possessions for individual health.[12] There is a lot of evidence that natural plants and other foods are a good antioxidants source.[13–16] *Tamarix gallica* belongs to family Tamaricaceae (known as Jhau in Hindi) is a herbaceous, deciduous, and perennial twiggy shrub that thrives in moist regions such as riverbanks, especially in salty soils.[17,18] The plant is rich in polyphenolic substances including flavonoids, phenols, saponins, tannins, coumarins, and terpenes.[19] Its major phytoconstituents are tamarixin, tamarixetin, quercetol, troupin, 3, 3-di-O-methylellagic acid, and 4-methylcoumarin.[18,20] *Tamarix gallica* has the reported hepatoprotective, antioxidant, anticancer, and antimicrobial activities.[17,21,22] The plant is traditionally used in leukoderma, spleen, and liver disorders. This study was proposed to reveal the antioxidant and antidiabetic properties of the *Tamarix gallica.*

Materials and methods

Plant procurement

Tamarix gallica was obtained from domestic market Lahore, Pakistan. Then, expert botanists from the Botany Department of Government College University Faisalabad, Pakistan, acknowledged it.

Extract preparation

Extraction procedure as described by Mustafa *et al.*[13] was used to prepare the extracts. Following a distilled water rinse, the plant was dried under shade and powder was prepared. The powder (50 g) was then steeped in ethanol, methanol, and distilled water (each 250 mL) for 72 h, shaking and mixing occasionally. Filter paper was used to filter the mixture (Whatman No. 1). Filtrates were concentrated and transferred to a petri plate in a rotary evaporator (SCI100-Pro; SCILOGEX, USA) at 40°C. The petri plate was stayed in an incubator at 40 C until it had dried out completely. Up until further research, the extract was kept at 4°C.

Qualitative phytochemical analysis

According to Singh and Bag,[23] phytochemical studies of extracts were carried out qualitatively using established procedures to determine the key phytochemical components.

Quantitative phytochemical assessment

Total phenolic contents (TPC)

10 µL of plant extract (1 mg/mL) were diluted in Folin-Ciocalteau reagent (100 µL) and 2.5% Na_2CO_3 (200 µL). TPC in the extracts was measured by mean of the gallic acid standard curve, as described by Kainama *et al.*[24] Absorbance (A) was measured after incubation (60 min) at room temperature by mean of biochemistry analyzer (Biolab-310) at 760 nm. TPC was measured in mg GAE/g of TPC.

Total flavonoid contents (TFC)

TFC were calculated using Quercetin (Q) as a standard, as described by Bajalan *et al.*[25] In a nutshell, 1 mL of distilled water (DW) was mixed with 100 µL of plant extract (1 mg/mL). After 5 min of room temperature incubation, 125 µL of aluminum chloride and 75 µL of 5% sodium nitrite were added and incubated for another 6 min at room temperature. Finally, 1M sodium hydroxide (125 µL) was mixed, and the absolute volume was made with DW up to 2.5 mL. Using a chemical analyzer, the absorbance was estimated at 540 nm (Biolab-310).

Antioxidant assay

Ferric reducing antioxidant potential (FRAP) assay (µmole Fe^{2+}/g DW)

FRAP was determined by the method as verified by Sethi *et al.*[26] 3.995 mL working solution (10 mM 2, 4, 6-tripyridyl-s-triazine (one volume) in 40 mM HCl, 300 mM acetate buffer (10 volumes), 20 mM ferric chloride (one volume) was mixed together with the 5 µL sample. The absorbance was taken at 593 nm.

ABTS assay (Trolox equivalent/g DW)

With minor modifications, the ABTS test was carried out as demonstrated by Asem *et al.*[27] The ABTS combination was prepared by mixing a 7-mM ABTS solution in distilled water with a 1:1 ratio of $K_2S_2O_8$ solution (2.5 mM). To get an absorbance of 0.7 at 734 nm, the resulting mixture was further diluted with methanol. After that, each plant extract solution (5 µL) was combined with ABTS solution (3.995 mL). After incubation (30 min) at room temperature, the absorbance was taken at 734 nm.

DPPH scavenging activity (percent inhibition)

Dimethyl sulfoxide (DMSO) was used to dilute the plant extracts at mg/mL concentration. In methanol (0.025 g/L), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was dissolved. 5 µl of sample solution were combined with 585 µL of DPPH working solution. Using a chemical analyzer (Biolab-310), the absorbance was measured at 515 nm after a 20-min incubation period at room temperature. By mean of the following equation, DPPH scavenging activity percentage was calculated:

A0–A1/A0100 = % DPPH scavenging activity

A0 is the absorbance of the control (the sample was changed with DW) and A1 is the absorbance of the sample.[28]

In vitro antidiabetic assay

α-amylase inhibitory activity

500 µL of plant extract (25, 50, 75, and 100 mg/mL dH2O) and 500 µL amylase solution (0.5 mg/mL in phosphate buffer;

pH 7.4) were kept at room temperature for 10 minutes. Then, in 0.02M sodium phosphate buffer, a 1% starch solution (500 µL) was mixed (pH 7.4). After incubation (10 min) at room temperature, the reaction was ended by mixing 1mL of 3,5-dinitrosallicylic acid (DNSA) color reagent. After 10 min in a boiling water bath, the mixture was cooled to room temperature and diluted with 10 mL DW. A biochemistry analyzer (Biolab-310) was used to measure absorbance at 540 nm.

For α-amylase, the percent of inhibition was calculated as follows

$$\text{Percent inhibitory activity of } \alpha\text{-amylase} = \frac{A_0\text{-}A_1}{A_0 \times 100}$$

A_0 is the absorbance of the control (extract sample was substituted with D.W) and A_1 is the absorbance of the sample.[29]

α-glucosidase inhibitory activity

500-µL plant extract (25, 50, 75, and 100 mg/mL dH2O), 1% starch solution (500 µL) in 0.2M Tris buffer (pH = 8) and 500-µL glucosidase solution (1 U/mL in tris-buffer; pH 8) were maintained at 37°C for 10 min. To stop the process, the solution was placed for 2 min in a boiling water bath. The amount of glucose released is calculated. A blank sample, which does not include the test sample, represents 100% enzyme activity. As a positive control, acarbose (glucosidase inhibitor) was used. A biochemistry analyzer (Biolab-310) was used to detect absorbance at 540 nm.

For α-glucosidase, the percent of inhibition was calculated as follows

$$\text{Percent inhibitory activity of } \alpha\text{-glucosidase} = \frac{A_0\text{-}A_1}{A_0 \times 100}$$

A_0 is the absorbance of the control (extract sample was substituted with DW) and A_1 is the absorbance of the sample.[29]

Evaluation of antioxidative enzymes

Catalase (CAT) assay

The previously reported method was followed to measure the activity of the Catalase enzyme in a 96-well plate containing 12 mM KH_2PO_4 (pH 7.0), 31.25 mM H_2O_2 and secretome in all the experimental wells containing blank wells, metformin, EthTg, MthTg, and AqTg. After light exposure for 30 to 60 s, absorbance was measured at 240 nm against a blank. The difference in absorbance at 45 and 60 s was used to calculate CAT activity. The enzyme activity was calculated from the difference of absorbance at 45 and 60 s and expressed as enzyme units per gram of fresh weight (Ug^{-1} FW). All the procedures were repeated thrice.[30]

Superoxide dismutase (SOD) assay

The previously reported method of Shamim and Rehman[30] was followed to estimate the activity of SOD enzyme in 96-well plate having the HepG2 cells. The reaction solution contained secretome for various treatment groups and mixed with 100 mM of KH_2PO_4 buffer with a pH of 7.8, ethylenediaminetetraacetic acid (EDTA) (0.1 mM), methionine (13 mM), nitro-blue tetrazolium chloride (NBT; 2.25 mM), and riboflavin (60 µM). This mixture, along with metformin and different concentrations of various plant extracts, were added to the HepG2 cell. All the wells except blank were exposed to light for 10 min, and absorbance was taken at 560 nm. The final value for SOD activity was calculated using the following equation and expressed as units per gram of fresh weight (Ug^{-1} FW). All the procedures were repeated thrice.[30]

SOD Activity = Absorbance of Control − Absorbance of sample

Cell line and glucose uptake method

The HepG2 cell lines were cultured in a culture flask containing Dulbecco's Modified Eagle Medium (DMEM) solution supplied with 2% of fetal bovine serum (FBS), penicillin G (100 mg/mL; Sigma) and streptomycin (100 U/mL; Sigma) for 5 days. The flask was placed in humidified CO_2 incubator at 37°C and 5% CO_2. Experiments were performed thrice in replicates. The cells were seeded to confluence into a 96-well plate and three wells were left as blank. The DMEM was then removed and replaced by RPMI 1640 having 2 g/L of glucose supplied with 0.2% bovine serum albumin. After 2 h, the medium was removed and replaced with the fresh medium again to treat the cells with various plant extracts EthTg, MthTg, and AqTg in different concentrations (5.0, 2.5, 1.25, 0.63, and 0.312 mg/mL) and 0.01 mM metformin. DMSO was added to the first three wells used as blank. After 2 days of treatment, the concentration of glucose in the medium was measured by the glucose-oxidase method.[31,32]

Statistical analysis

All of the measurements were done in duplicate. Using SPSS-23, the attained data was analyzed using one-way analysis of variance (ANOVA) and Tukey's post hoc test for comparison of mean values. All of the data were presented as a mean ± standard deviation.

Results

Qualitative phytochemical evaluation

The phytochemicals (such as flavonoids, alkaloids, carbohydrates, phenols, steroids, saponin, reducing sugar, terpenoids, and tannins) presence or absence in MthTg, EthTg, and AqTg is shown in Table 1.

Total flavonoid and phenolic contents

TFC and TPC were significantly ($p = 0.05$) higher in EthTg (16.24 ± 1.14 mg QE/g and 17.4 ± 0.41 mg GAE/g, respectively) than MthTg (9.86 ± 1.78 mg QE/g and 15.02 ± 1.74 mg GAE/g, respectively) and AqTg (8.3 ± 0.48 mg QE/g and 7.59 ± 1.42 mg GAE/g, respectively) as revealed in Figure 1.

In vitro antioxidant assessment

The obtained data of FRAP, ABTS, and DPPH• (percent inhibition) assay are described in the Figures 2 and 3. Figure 2 showed EthTg has the highest Fe^{3+} into Fe^{2+}

Table 1. Qualitative phytochemical evaluation of *Tamarix gallica.*

Compounds	Test	EthTg	MthTg	AqTg
Carbohydrates	Benedict's test	+++	++	++
	Fehling's test	–	–	–
Reducing sugar	Fehling's test	–	–	–
Alkaloids	Hager's test	++	++	++
Proteins	Xanthopeoteic test	+++	++	++
Flavonoids	Alkaline reagent test	+++	+++	++
Phenols	Lead acetate test	+++	+++	++
Tannins	Lead acetate test	+	+	+
Steroids	Salkowski's test	–	–	–
Terpenoids	Salkowski's test	–	–	–

(+): present; (–): Absence.
EthTg: Ethanol extract of *Tamarix gallica*; MthTg: Methanol extract of *Tamarix gallica*; AqTg: Aqueous extract of *Tamarix gallica.*

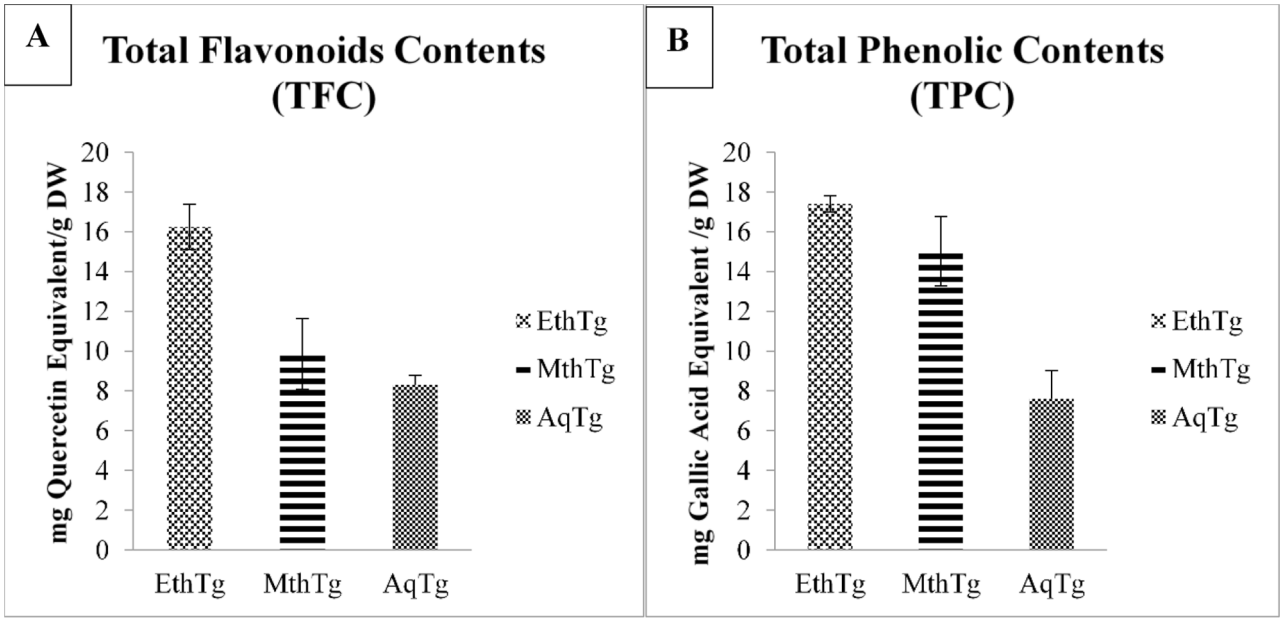

Figure 1. (A) TFC of Tamarix gallica arial part extracts. (B) TPC of Tamarix gallica arial part extracts.
Results are presented as mean ± standard deviation of three replicates of each extract, that is, EthTg (ethanolic extract of *Tamarix gallica*), MthTg (methanolic extract of *Tamarix gallica)*, and AqTg (aqueous extract of *Tamarix gallica*). DW refers to the dry weight of *Tamarix gallica* extract.

reducing potential (323.51 ± 2.32 µmole Fe^{2+}/g) than MthTg (309.82 ± 13.9 µmole Fe^{2+}/g) and AqTg (245.69 ± 8.87 µmole Fe^{2+}/g). Parallel trend was detected in ABTS scavenging radical being highest in EthTg (266.97 ± 25.14 µM TE/g) than MthTg (259.39 ± 11.15 µM TE/g) and AqTg (240.08 ± 4.47 µM TE/g) (Figure 2(B)). For DPPH activity, the absorbance was taken at five different concentrations (0.312, 0.625, 1.25, 2.5, 5). Figure 3 indicated a concentration-dependent rise in DPPH scavenging activity in every extract. EthTg which has the lowest inhibitory concentration (IC50) (1.309 ± 0.31 mg/mL) than MthTg (1.606 ± 0.43 mg/mL), and AqTg (1.911 ± 0.61 mg/mL) showed maximum DPPH scavenging activity.

Catalase and SOD

Results showed catalase and SOD activities was increased in EthTg on different concentrations as compared to MthTg and AqTg extracts. Table 2 showed the catalase and SOD activities of EthTg, MthTg and AqTg extracts on different concentrations. EthTg also showed more catalase and SOD activities as compared to control group.

In *vitro* antidiabetic assessment

α-amylase inhibitory activity

In α-amylase inhibitory activity of all *Tamarix gallica* extracts, a concentration (0.312, 0.625, 1.25, 2.5, and 5) dependent increase has been noticed (Figure 4(A)). Percentage inhibition of α-amylase inhibitory activity was increased in each extract as concentration increased. EthTg revealed the highest α-amylase inhibitory activity as to have lower IC50 value (1.116 ± 0.051 mg/mL) than MthTg (1.67 ± 0.09 mg/mL) and AqTg (2.002 ± 0.26 mg/mL). Acarbose showed the lower IC50 value (0.35 ± 0.03 mg/mL) as compared to all extract types (Figure 4(B)).

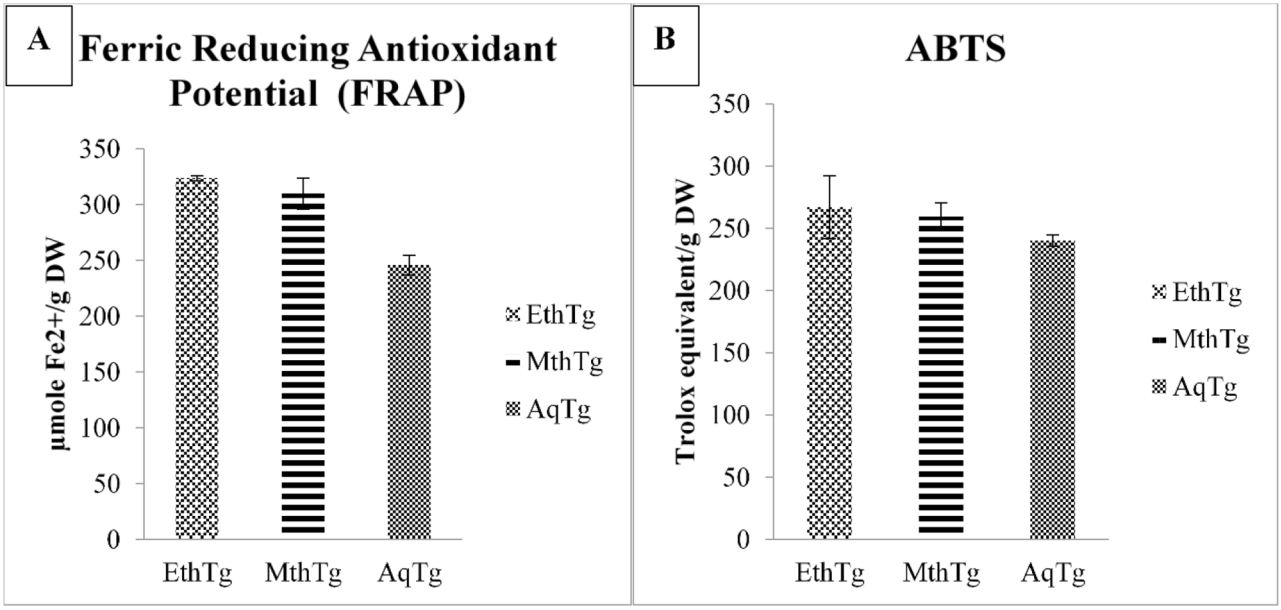

Figure 2. (A) FRAP of *Tamarix gallica* arial part extracts. (B) ABTS assay of *Tamarix gallica* arial part extracts.
Results are presented as mean ± standard deviation of three replicates of each extract, that is, EthTg (Ethanolic extract of *Tamarix gallica*), MthTg (Methanolic extract of *Tamarix gallica)*, and AqTg (aqueous extract of *Tamarix gallica*). DW refers to the dry weight of *Tamarix gallica* extract.

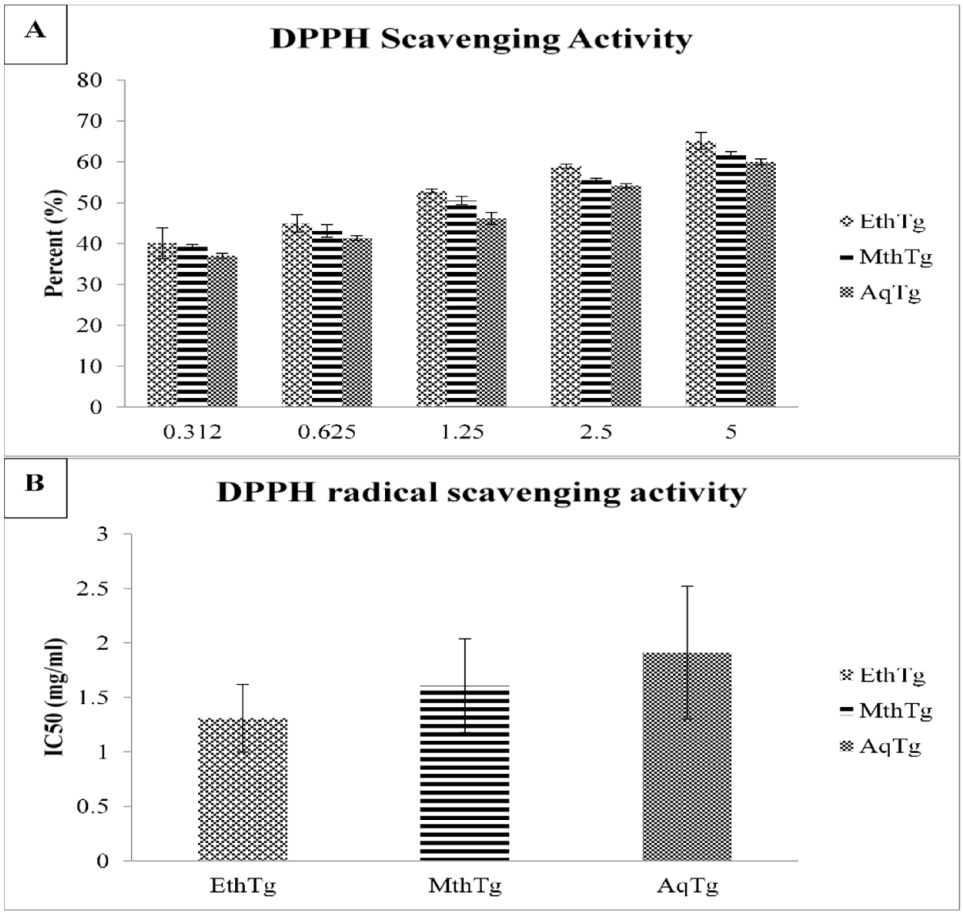

Figure 3. (A) DPPH scavenging activity of five different absorptions of *Tamarix gallica* arial part extracts. (B) DPPH IC_{50} value of *Tamarix gallica* arial part extracts.
Results are presented as mean ± standard deviation of three replicates of each extract, that is, EthTg (Ethanolic extract of *Tamarix gallica*), MthTg (Methanolic extract of *Tamarix gallica)*, and AqTg (aqueous extract of *Tamarix gallica*).

Table 2. Catalase activity, superoxide dismutase activity and glucose consumption via cell line.

Groups	Concentration	Catalase activity (U g^{-1} FW)	SOD activity (U g^{-1} FW)	Glucose consumption (µg/mL)
Control group (CG)	0	0.43 ± 0.01^{b}	0.483 ± 0.012^{b}	1.74 ± 0.02^{d}
Standard group (SG)	0.1 µM	–	–	5.21 ± 1.04^{a}
EthTg	5	0.76 ± 0.004^{a}	0.61 ± 0.0021^{a}	4.91 ± 0.12^{b}
	2.5	0.71 ± 0.012^{a}	0.57 ± 0.003^{a}	4.19 ± 0.73^{b}
	1.25	0.54 ± 0.05^{a}	0.51 ± 0.01^{a}	4.37 ± 0.49^{b}
	0.625	0.63 ± 0.09^{a}	0.54 ± 0.003^{a}	4.87 ± 0.7^{b}
	0.312	0.58 ± 0.034^{a}	0.56 ± 0.078^{a}	4.91 ± 0.32^{b}
MthTg	5	0.43 ± 0.071^{b}	0.41 ± 0.14^{b}	3.46 ± 0.59^{c}
	2.5	0.49 ± 0.014^{b}	0.37 ± 0.067^{b}	3.12 ± 0.41^{c}
	1.25	0.39 ± 0.1^{b}	0.45 ± 0.05^{b}	2.91 ± 0.37^{c}
	0.625	0.41 ± 0.04^{b}	0.31 ± 0.12^{b}	2.84 ± 0.78^{c}
	0.312	0.35 ± 0.14^{b}	0.39 ± 0.13^{b}	2.79 ± 0.81^{c}
AqTg	5	0.29 ± 0.03^{c}	0.2 ± 0.06^{c}	1.91 ± 0.04^{d}
	2.5	0.28 ± 0.061^{c}	0.15 ± 0.021^{c}	2.01 ± 0.12^{d}
	1.25	0.21 ± 0.032^{c}	0.17 ± 0.17^{c}	1.87 ± 0.23^{d}
	0.625	0.27 ± 0.12^{c}	0.22 ± 0.09^{c}	1.32 ± 0.42^{d}
	0.312	0.16 ± 0.19^{c}	0.14 ± 0.19^{c}	1.53 ± 0.1^{d}

SOD: superoxide dismutase; EthTg: Ethanol extract of *Tamarix gallica*; MthTg: Methanol extract of *Tamarix gallica*; AqTg: Aqueous extract of *Tamarix gallica*. Superscripts describe the significance of extracts at the different concentrations.

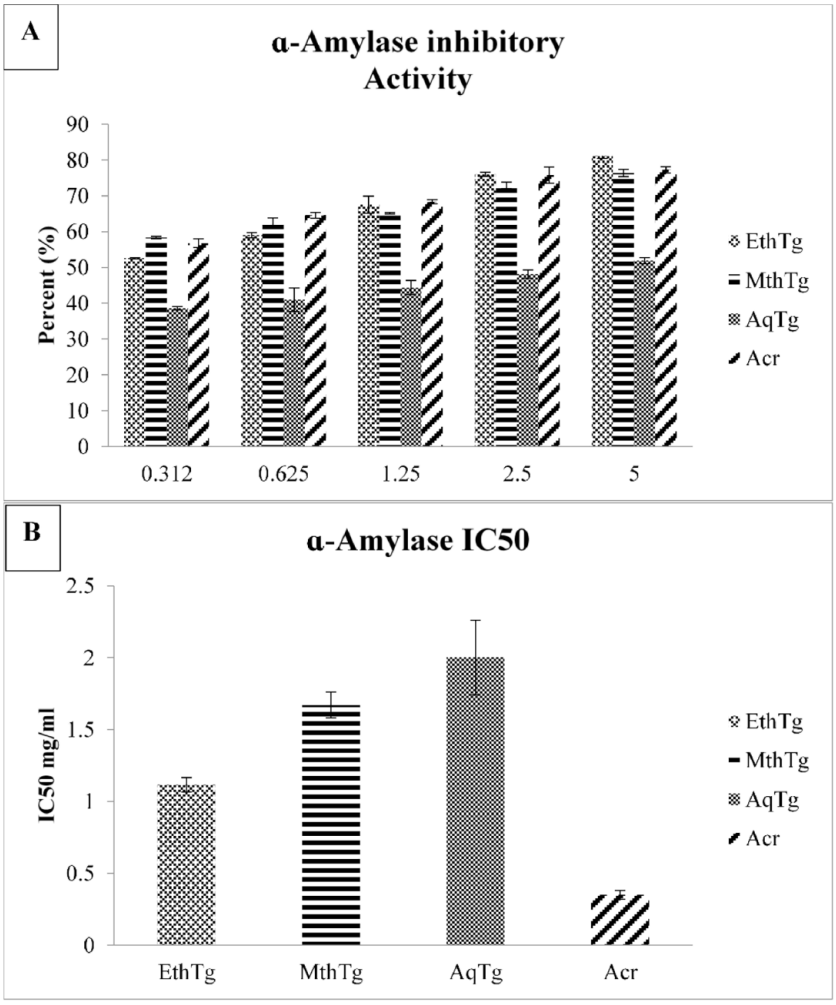

Figure 4. (A) α-Amylase inhibitory activity of five different absorptions of *Tamarix gallica* arial part extracts. (B) IC_{50} value of α-Amylase inhibitory activity of *Tamarix gallica* arial part extracts.
Results are presented as mean ± standard deviation of three-replicates of each extract, that is, EthTg (Ethanolic extract of *Tamarix gallica*), MthTg (Methanolic extract of *Tamarix gallica)*, and AqTg (aqueous extract of *Tamarix gallica*).

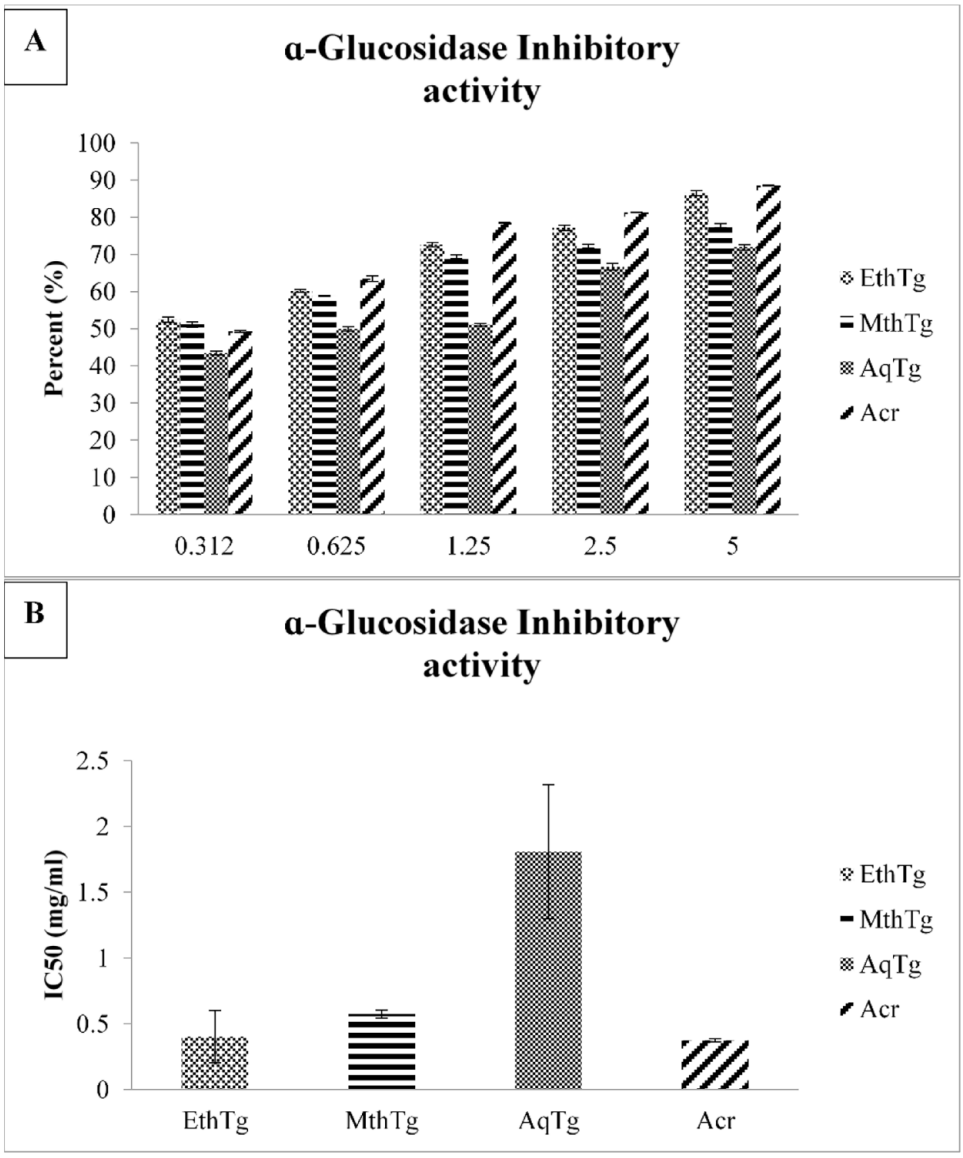

Figure 5. (A) α-Glucosidase inhibitory activity of five different absorptions of *Tamarix gallica* arial part extracts. (B) IC_{50} value of α-Glucosidase inhibitory activity of *Tamarix gallica* arial part extracts.
Results are presented as mean ± standard deviation of three-replicates of each extract, that is, EthTg (Ethanolic extract of *Tamarix gallica*), MthTg (Methanolic extract of *Tamarix gallica)*, and AqTg (aqueous extract of *Tamarix gallica*).

α-glucosidase inhibitory activity

α-glucosidase inhibitory activity of the all extracts, and acarbose revealed a concentration-dependent (0.312, 0.625, 1.25, 2.5, 5) rise in percent activity (Figure 5(B)). EthTg revealed the utmost α-glucosidase inhibitory activity with lowest IC50 value (0.402 ± 0.2 mg/mL) than MthTg (0.574 ± 0.03 mg/mL) and AqTg (1.807 ± 0.51 mg/mL). Acarbose showed the utmost α-glucosidase inhibitory activity with lowest IC50 value (0.375 ± 0.012 mg/mL) as compared to all extract types (Figure 5(B)).

Glucose uptake via cell line

Glucose consumption in HepG2 cell lines was high in EthTg on different concentrations as compared to MthTg and AqTg extracts. Table 2 showed the glucose consumption of EthTg, MthTg and AqTg extracts on different concentrations. EthTg showed more glucose consumption as compared to control group. Standard drug Metformin showed more glucose consumption as compared to EthTg extract.

Antioxidant activity correlation with phytochemicals

TPC and antioxidant assays such as ABTS (R2 = 0.995), FRAP (R2 = 0.998), and DPPH IC50 value (R2 = 0. 0.928) showed a strong correlation. (Table 3). TFC had also showed a good correlation with DPPH IC50 (R2 = 0.879). FRAP (R2 = 0.589) and ABTS (R2 = 0.696) also showed positive correlation with TFC but not strong (Table 3).

Antioxidants and phytochemicals correlation with antidiabetic assays

The correlation results of TFC and TPC with antidiabetic assay in context of α-amylase inhibition activity IC50 value (R^2 = 0.963, R^2 = 0.824 respectively) indicated that TFC and

Table 3. Correlation of phytochemicals in *Tamarix gallica* with antioxidant and antidiabetic parameters.

	Phytochemicals		Antioxidant activity			Antidiabetic activity	
	TPC	TFC	FRAP	ABTS	IC_{50} value of DPPH	IC_{50} value of α-amylase inhibitory activity	IC_{50} value of α-glucosidase inhibitory activity
Phytochemicals							
TPC	1	–	0.995**	0.992**	0.9283**	0.8243**	0.9851**
TFC	–	1	0.590**	0.6962**	0.8792**	0.963**	0.5371**
Antioxidant activity							
FRAP	0.995**	0.590**	1	–	–	0.7687**	0.9972**
ABTS	0.992**	0.6962**	–	1	–	0.8552**	0.9732**
IC_{50} value of DPPH	0.9283**	0.8792**	–	–	1	0.9741**	0.8532**
Antidiabetic activity							
IC_{50} value of α-amylase inhibitory activity	0.8243**	0.963**	0.7687**	0.8552**	0.9741**	1	–
IC_{50} value of α-glucosidase inhibitory activity	0.9851**	0.5371**	0.9972**	0.9732**	0.8532**	–	1

TPC: total phenolic contents; TFC: total flavonoid contents; FRAP: ferric reducing antioxidant potential; IC: inhibitory concentration; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid.
**Correlation is significant at ($p \leqslant 0.01$).

TPC are positively correlated with the alpha amylase inhibition activity. In context of α-glucosidase inhibition activity, TFC and TPC also presented positive correlation ($R^2 = 0.531$, $R^2 = 0.985$, respectively) (Table 3). All antioxidant parameters revealed positive correlation with antidiabetic assay (Table 3). IC_{50} value of α-amylase inhibitory activity showed highly positive correlation with DPPH assay ($R^2 = 0.9741$) as compared to FRAP ($R^2 = 0.7687$) and ABTS ($R^2 = 0.8552$) as described in the Table 3. However, IC_{50} value of α-glucosidase inhibition activity showed highly positive correlation with FRAP ($R^2 = 0.9972$) and ABTS ($R^2 = 0.9732$) as compared to DPPH assay ($R^2 = 0.8532$) (Table 3).

Discussion

It is well known that throughout history, people have utilized nature to satiate their basic needs. This also applies for the use of natural ingredients as medicines for a variety of illnesses.[33] Today, traditional medicines are used by between 70 and 95 percent of people in underdeveloped countries.[34] One of the main classes of secondary plant compounds is the phenols.[35] Unique phenolic content with antioxidant property can have a key importance in the free radicals neutralization and adsorption.[13,29,36] Effective therapeutic properties such as anticholinergic,[37] antibacterial,[38] antioxidant,[39] anticancer,[40] and antidiabetic[29] are present in these substances. Around 4500 known components are included in the secondary derivative flavonoids.[41] Long-established anti-inflammatory,[42] anti-diabetic,[43] antioxidant,[44] and anti-cancer[45] properties of flavonoids have positive impacts on health. This study evaluates the TFC and TPC in ethanolic, methanolic, and aqueous extracts of the *Tamarix gallica.* The study results exposed that EthTg showed maximum TFC and TPC as compared to the MthTg and AqTg (Figure 1). Recently, Elamin[46] revealed maximum flavonoids in the *Tamarix gallica* leaves extracted from ethyl acetate and n-butanol. Said *et al.*[47] also showed the presence of TFC and TPC in *Tamarix gallica.* In a previous study, Boulaaba *et al.*[48] evaluated five flavonoids in *Tamarix gallica* flower extracts including kaempferol and quercetin. Drabu *et al.*[49] found TFC and TPC in methanolic extract of *Tamarix gallica* arial part.

Oxidative alterations in important biomolecules, such as lipid peroxidation, carbonyl (ketone /aldehyde) adduct production, protein carbonylation, sulfoxidation, nitration, and DNA damage like strand breaks or nucleobase oxidation, are linked to oxidative stress.[50] It will be necessary to attain redox status equilibrium if the antioxidants made endogenously are unable to stop the synthesis of reactive species. Plants, which contain natural antioxidants, have a big impact in this particular circumstance.[51,52] In the present study, antioxidant property of the *Tamarix gallica* extracts were exposed by using ABTS, DPPH, and FRAP methods. The obtained data showed the substantial antioxidant outcome of the plant extract. FRAP and ABTS data shown that EthTg possess the highest antioxidant ability as compared to MthTg and AqTg (Figure 2). Similar pattern was seen in DPPH result as EthTg showed maximum antioxidant activity with lowest IC50 as compared to MthTg and AqTg (Figure 3). On cell line, catalase and SOD activities also showed EthTg has the maximum antioxidant activity as compared to MthTg and AqTg. Recently, Lefahal *et al.*[53] also revealed the antioxidant activity of methanol and ethyl acetate extracts of the *Tamarix gallica* in context of DPPH and TAC assay. Both extracts showed strong TAC (methanol = 287.01 ± 7.85, ethyl acetate = 246.7 ± 1.12 mg AAE/g) and DPPH (IC50 methanol = 14.05 ± 0.66, IC50 ethyl acetate = 27.58 ± 1.98 μg/mL) activity. Bettaib *et al.*[22] revealed *in vitro* (Fe-reducing power, $EC_{50} = 100\ \mu g\ mL^{-1}$; ABTS test, $IC_{50} = 50\ \mu g\ mL^{-1}$; DPPH assay $IC_{50} = 6\ \mu g\ mL^{-1}$) and *in vivo* antioxidant activity of *Tamarix gallica* against H_2O_2 exposed small intestine epithelial cells of rats. *Tamarix gallica* improved the CAT, SOD and MDA levels against H_2O_2 causing oxidative stress.

In this study, antidiabetic property of *Tamarix gallica* was determined by revealing α-amylase and α-glucosidase inhibitory activity of the plant. Key enzymes in the digestion of starch and glycogen are α-amylase and α-glucosidase,[54] which play important roles in regulating the glucose content.[55] The study obtained data revealed that all extract

have the good antidiabetic property. EthTg had the utmost α-amylase inhibitory activity with lowest IC_{50} value as compared to MthTg and AqTg (Figure 4). Likewise, EthTg also showed utmost α-glucosidase inhibitory activity than MthTg and AqTg (Figure 5). On cell line, glucose uptake was also high in EthTg as compared to MthTg and AqTg on concentration depended manner. Malik *et al.*[56] revealed antidiabetic property of *Tamarix gallica* against alloxan induced hyperglycaemia. Because of its alpha amylase and anti-oxidant potential, the aerial parts of *Tamarix gallica* have a higher anti-diabetic profile.[56] Glucuronosylated and O-Methylated flavonoids from *Tamarix gallica* increase α-glucosidase inhibitory activity.[57]

Numerous research works support the strong relationship between TPC and TFC's antioxidant properties.[13,29,58] The findings of this study also demonstrate a close relationship between TPC and TFC and antioxidant activities as measured by the ABTS, FRAP and DPPH assays. It also demonstrates that elevated TPC and TFC levels are the cause of elevated DPPH activity of EthTg. The DPPH IC50 and TPC have a high connection ($R^2 = 0.928$), indicating that increasing the TPC has increased the DPPH scavenging property (Table 3). Similar strong correlation of TPC with FRAP ($R^2 = 0.998$) and ABTS ($R^2 = 0.995$) was seen. TFC also showed positive correlation with all antioxidant parameters (Table 3). Recently, Lefahal *et al.*[53] demonstrated that antioxidant activity of *Tamarix gallica* extracts were possibly associated to polyphenols content and flavonoids. TPC and TFC correlation with antidiabetic activity indicated that if these contents were elevated, α-amylase ($R^2 = 0.824$ and $R^2 = 0.963$ respectively) and α-glucosidase inhibitory activities ($R^2 = 0.985$ and $R^2 = 0.531$, respectively) also be elevated. Antioxidant parameters (ABTS, FRAP, and DPPH) also showed positive correlation with antidiabetic parameters (α-amylase inhibitory activity and α-glucosidase inhibitory activity) (Table 3). Mustafa *et al.*[13] also showed the positive correlation of phenolic contents with antioxidant and antidiabetic activities.

Conclusions

Tamarix gallica ethanolic extract had the strongest antioxidant capacity among other extracts as well as had high TFC and TPC. In terms of inhibiting α-glucosidase and α-amylase, maximum antidiabetic efficacy was also shown by ethanolic extract. TFC and TPC had positive correlation with antioxidant and antidiabetic activities. Antioxidant activity also directly proportional to antidiabetic activity. So, it is concluded that *Tamarix gallica may have the tendency to manage diabetes and oxidative stress related diseases.*

AUTHORS' CONTRIBUTIONS

JN, SMAS, SA, and MA designed and conducted the study; JN, AR, and IM analyzed the data; JN, IM, and ZN review and wrote the manuscript.

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