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Phenolic profile and antioxidant activity of *Tribulus terristris* polyphenols

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Abstract

The aim of this study was identification and quantification of flavonoids and phenolic acids from various *T. terristris* (TT) extracts and evaluation of antioxidant and free radical scavenging activities. Different solvent systems were used to prepare TT extracts of different polarities. Maximum extract yield (14.32 g/100g of dry material) was recorded with 80% methanol solvent. Reverse phase high performance liquid chromatography (RP-HPLC) analysis of TT extracts revealed the presence of syringic acid, salicylic acid and gallic acid as major phenolic acids and catechin, myricetin, quercetin and Kaempferol as major flavonoids. Total phenolic (TP) and total flavonoid (TF) contents of TT extracts were measure as gallic acid equivalent (GAE) and catechine equivalent (CE), respectively. The significant variations ($P< 0.05$) in the amounts of TP and TF contents were observed in TT extract of different solvents. Maximum TP (38.01 mg/g of dry material, measured as GAE) and TF (11.91 mg/g of dry material, measured as CE) contents were recorded in 80% methanol extract of TT. Hexane extract showed the least TP and TF contents. The FA extracts showed excellent DPPH radical scavenging activity (IC_{50}= 11.35 µg/ml), Inhibitation of linoleic acid peroxidation (89.40%), reducing power. The results are comparable with the synthetic antioxidants. The results showed the potential of TT as source of natural antioxidants.

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Introduction

High level of free radicals and active oxygen species (ROS) create oxidative stress, which leads to a variety of biochemical and physiological lesions and often results in metabolic impairment and cell death (Ames, 1998). The most ROS include superoxide anion, hydrogen peroxide (H_2O_2), peroxyl (ROO-) radicals, and reactive hydroxyl (OH) radicals.

Epidemiological evidences indicate that the consumption of food stuffs containing antioxidant phytochemical (notably flavonoids and other polyphenols) is advantageous for our health (Cao *et al*., 1996; Di Carlo *et al*., 1999; Pulido *et al*., 2000), since they can protect the human body from free radicals and retard the progress of many chronic diseases. For this reason, the development and isolation of natural antioxidants from plant species are in progress.

Tribulus terrestris is a well-known and widely distributed species of the genus *Tribulus*. It is known with several common names: puncture vine, caltrop, goat head, bull's head, ground burr nut, devil's thorn (Kostova and Dinchev, 2005) and Arabic names: Al-Gutub, Qutiba, Hasak or DersEl-Agouz (Al-Ali *et al*., 2005). It is popularly claimed to improve sexual function possibly through increase in the free serum testosterone (Brown *et al*., 2001).

Anthelmintic (Deepak *et al*., 2002) antifungal (Bedir *et al*., 2002; Zhang *et al*., 2006) and antibacterial (Ali *et al*., 2001) activities have also been reported in *T. terrestris* extracts. Steroidal saponins from *T. terrestris* have been reported to possess anticancer activity (Bedir *et al*., 2002; Kumar *et al*., 2006) and to protect hepatocytes from cell death (Li *et al*., 1998).

As a part of our on-going investigations on natural antioxidants, the present study was designed to investigate the phenolic contents of *T. terrestris* plant and identification of antioxidant compounds from different extract of TT and evaluation of antioxidant and free radical scavenging activities.

Materials and methods

Collection, identification and pre-treatment of plant materials

Whole plants of *T. terristris* were collected from the Bakhar Punjab, Pakistan in April-May. The plant specimen was identified and authenticated by Dr. Muhammad Qasim (Taxonomist), Department of Botany, Government College University, Faisalabad, Pakistan. The roots were separated from the plants and the remaining plant materials were dried at 35 ^{o}C and milled (Tector-Cemotec 1090 sample mill, Hognas, Sweden). The milled plant materials were sieved and fractions that were sieved through a 70-mesh (210 µm) sieve and retained on an 80-mesh (177 µm) sieve were selected for extraction

Reference compounds, reagents and chemicals

Standards and reference chemicals used in this study including gallic acid, chlorogenic acid, ferulic acid, vanillic acid, *p*-coumeric acid, sinapic acid, *p*-hydroxy benzoic acid, caffeic acid, quercetin, myricetin, kaempferol, catechin, ascorbic acid, linoleic acid (60-74%), Folin-Ciocalteu reagent, 2,2-diphenyl- 1-picrylhydrazyl radical (DPPH), Tween 40, butylatedhy droxytoluene (BHT), butylatedhy droxylanisole (BHA) were obtained from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals (analytical grade) i.e. ferrous chloride, ammonium thiocyanate, hydrochloric acid, chloroform, hexane, ethanol, and methanol used in this study were purchased from Merck (Darmstadt, Germany), unless stated otherwise.

Sample Preparation

The grounded plant material of *Tribulus terristris* was defatted with *n*-hexane using soxhlet extraction apparatus. Absolute ethanol, absolute methanol, aqueous ethanol (80:20 v/v), aqueous methanol (80:20 v/v) and hexane extracts from the defatted plant material were obtained as described earlier (Hussain *et al*., 2013). Briefly, the ground plant material (200 g) was extracted with 700mL of solvents in a Soxhlet unit (1000mL capacity) for 18h. The extract was then filtered through Whatman filter paper (No. 1). The solvents were removed under reduced pressure, using a rotary evaporator (EYELA,

SB-651, Rikakikai Co. Ltd. Tokyo, Japan). The dried, crude concentrated extracts were weighed to calculate the yields and stored in a refrigerator (-4°C), until used for analyses.

HPLC analysis of phenolic acids and flavonoids

Hydrolysis of sample

The hydrolysis of different extracts were done as reported previously(Hussain *et al.*, 2013). Briefly, 10ml of 50% aqueous methanol solution containing 1.2 M HCl and 0.04% (w/v) ascorbic acid as antioxidant was added to 1000mg of crude extracts.

The hydrolysis was performed at 80°C under reflux for 2h. After refluxing, the extracts were allowed to cool and were made up to 10ml with methanol. The extracts were filtered through 0.45µm non-pyrogenic filter (Minisart, Satorius Stedim Biotech GmbH, Goettingen, Germany) prior to injection.

Preparation of calibration curves

Stock solutions of the standards (gallic acid, *p*-hydroxy benzoic acid, chlorogenic acid, caffeic acid, vanillic acid, *p*-coumeric acid, sinapic acid, ferulic acid, catechin, myricetin, quercetin, kaempferol) were freshly prepared by dissolving authentic compounds in methanol (100 µg/mL). Working standards solutions were made by gradual dilution with methanol to the required concentration 0.4 to 100 µg/mL. The calibration curve was constructed for each standard by plotting the concentration of standard against peak area.

Chromatographic conditions

The HPLC analysis was performed with Shimadzu CBM-20A system (Shimadzu Corporation, Kyoto, Japan) equipped with gradient model LC-20AD pumps system, a SPD 20A UV/Visible detector, CTO-10AS VP column oven, an auto injection (SIL-20AHT) and degasser (DGU-20A5) systems.

A hypersil GOLD C_{18} column (250x4.6mm internal diameter, 5µm particle size) (Thermo Fisher Scientific inc) and a non-linear gradient consisting of solvent A (acetonitrile:methanol, 70:30) and solvent B (water with 0.5% glacial acetic acid).

Following gradient program was used for the separation of phenolic acids and flavonoids; 10-15% A from 0 to 5min; 15-20% A from 5 to 18min; 20-40% A from 18 to 40min and kept at 40% A from 40 to 45 min; 40-10% A from 45 to 50 min and kept at 10% A from 50 to 55min). UV spectra were recorded at 275nm.

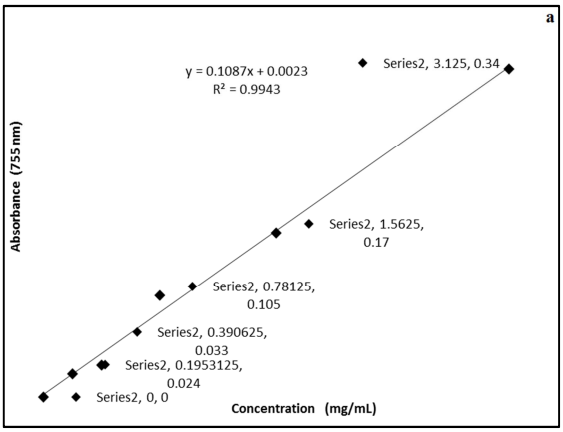
The analytes were identified by matching the retention times and spiking samples with standards and quantification was based on an external standard method. HPLC separation efficiency was assessed by the separation factor (α) and resolution (Rs). The reproducibility of each compound was measured and the standard deviation was calculated from six measurements through run-to-run and day-to-day basis.

Evaluation of in-vitro antioxidant activity

Determination of total phenolics (TP) contents

Amounts of TP from different solvents extracts were assessed using Folin-Ciocalteu reagent, reported previously (Hussain *et al.*, 2013). Briefly, 50mg of each crude extract was mixed with 0.5 mL of Folin-Ciocalteu reagent and 7.5mL deionized water.

The mixture was kept at room temperature for 10min, and then 1.5 mL of 20% sodium carbonate (w/v) was added. The mixture was heated in a water bath at 40°C for 20min and then cooled in an ice bath. Absorbance was measured at 755nm using a spectrophotometer (Bio Tek Instrument, Inc., VT, USA). Amounts of TP were calculated using gallic acid calibration curve (0.195-3.125 mg/mL) (Fig. 1a) and reported in mg/g of dry plant material, measures as gallic acid equivalent (GAE).



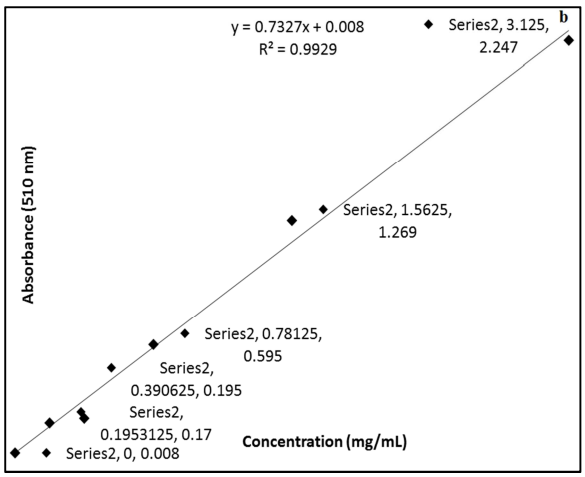


Fig. 1. Calibration curves of gallic acid (a) and catechin (b).

Determination of total flavonoids (TF) contents

Total flavonoid contents of TT extracts were determined following the procedure reported previously (Hussain *et al.*, 2013). Briefly, extract solution (1mL) containing 10mg extract was placed in a 10mL volumetric flask and then 5mL of distilled water was added followed by 0.3mL of 5% $NaNO_2$. After 5min, 600µL of 10% $A1C1_3$ was added. After another 5min 2mL of 1 M NaOH was added and volume was made up to 10mL with distilled water. Absorbance was measured at 510nm using spectrophotometer (Bio Tek Instrument, Inc, VT, USA). Total flavonoid contents were calculated using a calibration curve for catechin (0.195-3.125mg/mL) (Fig. 1b). The amounts of TF were calculated and reported in mg/g of dry plant material, measured as catechin equivalent (CE).

DPPH radical scavenging assay

2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay was carried out to measure the free radical scavenging activity as described earlier (Hussain *et al.*, 2013). The fractions and pure phenolic acid and flavonoids compounds concentrations in methanol (1-100g/mL) were mixed with 2mL of 90µm methanol solution of DPPH. After 30 minutes incubation period at room temperature, the absorbance was read at 517nm. BHT and BHA were used as positive control for comparison and 90M DPPH solution was taken as blank. The percent scavenging was calculated by following formula; Scavenging(%)= 100 x (A_{blank} - A_{sample}/A_{blank})

Where A_{blank} is the absorbance of the DPPH solution and A_{sample} is the absorbance of the extract solution. Extract concentration providing 50% scavenging (IC_{50}) was calculated from the graph-plotted inhibition percentage against extract concentration.

Reducing power

The reducing powers of extracts were determined according to the procedure reported earlier, with little modification (Anwar *et al.*, 2009). Briefly, concentrated extract (0.625–10.0mg) was mixed with sodium phosphate buffer (5.0mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0mL, 1.0%); the mixture was incubated at 50°C for 20min. Then 5mL of 10% trichloroacetic acid was added and the mixture centrifuged at 980×g for 10 min at 5°C in a refrigerated centrifuge (CHM-17; Kokusan Denki, Tokyo, Japan). The upper layer of the solution (5.0mL) was decanted and diluted with 5.0 mL of distilled water and ferric chloride (1.0mL, 0.1%), and absorbance read at 700 nm using spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). BHT and BHA were used as positive control (Hussain *et al.*, 2013).

Inhibition of linoleic acid peroxidation

The antioxidant activity of T.T different solvents extracts and pure compounds were also determined in terms of measurement of percent inhibition of linoleic acid peroxidation following a method reported before (Hussain *et ali.*, 2013). Briefly, 5mg of each extract and pure compounds was added to a solution mixture of linoleic acid (130°L), 99.8% ethanol (10 mL) and 10mL of 0.2m sodium phosphate buffer (pH 7). Total mixture was diluted up to 25 mL with distilled water. The solutions were incubated at 40°C for 175h and the degree of oxidation was measured before and after incubation, following thiocyanate method. Briefly, 10 mL of ethanol (75%), 200L of an aqueous solution of ammonium thiocyanate (30%), 200°L of sample solution and 200L of ferrous chloride ($FeCl_2$) solution (20mm in 3.5% HCl) were mixed sequentially. After 3min of stirring, the absorption values of the mixtures were determined at 500 nm. A negative control was performed with linoleic acid but without extracts. BHT and BHA were used as positive control.

Increases in absorbance values of samples, negative and positive controls were calculated by subtracting first value (0 h) from second value (175h) and inhibition of linoleic acid peroxidation was calculated using following formula;

Percent inhibition = 100 - [(Abs. increase of sample/Abs. increase of negative control) ×100]

Bleaching ability of -carotene-linoleic acid

Antioxidant activity of different *T. terristris* extracts were also assessed by measuring the bleaching of β-carotene/linoleic acid emulsion system as described by Hussain *et al.,* (2011) with modification. A stock solution of β-carotene-linoleic acid mixture was prepared by dissolving 0.1mg 3- carotene, 20mg linoleic acid, and 100mg Tween 40 in 1.0mL of chloroform (HPLC grade). The chloroform was removed under vacuum in rotary evaporator at 50°C. Then, 50mL of distilled water saturated with oxygen (30min, 100mL min^{-1}) were added with vigorous shaking. A 5.0mL of this reaction mixture was dispensed to test tubes with 200 µL of *T. terristris* extracts prepared at 4.0gL^{-1} concentrations and the absorbance was immediately measured at 490nm against a blank, consisting of an emulsion without β-carotene. Then emulsion was incubated for 50h at room temperature and the absorbance was recorded at different time intervals. The same procedure was repeated with BHT and blank.

Statistical Analysis

All the experiments were carried out in triplicate and the data are presented as mean values ± standard deviation (SD). Statistical analysis of the data was performed by Analysis of Variance (ANOVA) and Duncan's multiple range tests using STATISTICA 5.5 (Stat Soft Inc, Tulsa, Ok, and USA) software and a probability value of $P \leq 0.05$ was considered to represent a statistical significance difference among mean values.

Results and discussion

Extracts yields

The yields of absolute ethanol, absolute methanol, 80% ethanol, 80% methanol and hexane extracts of *T. terristris* are given in Table 1. The amount of components extracted from different parts of *T. terristris* plant using different solvents varied widely. Generally, the highest extract yield was obtained with aqueous methanol (14.32 g/100g of dry sample) followed by aqueous ethanol (11.04 g/100g of dry sample), absolute ethanol (9.21 g/100g of extract) and absolute methanol (8.54 g/100g of dry sample). Lease extract yield (2.13 g/100g of dry samples) was recorded with hexane. Methanol is generally employed for the extraction of antioxidants components from plant materials due to their polarity and good solubility with many phenolic active components (Siddhuraju & Becker, 2003).

Table 1. Extract yield (g/100g) of *Tribulus terristeris* using different solvent systems.

Solvent Systems	Extract Yield (g/100g)
Absolute Methanol	8.54 ± 0.62 ^{b}
Aqueous Methanol	14.32 ± 1.09 ^{d}
Absolute Ethanol	9.21 ± 0.72 ^{b}
Aqueous Ethanol	11.04 ± 0.50 ^{c}
Hexane	2.13 ± 0.10 ^{a}

Values are mean ± standard deviation of three independent experiments. Values with different letters in superscript represent significant difference ($p< 0.05$) among different solvent systems.

HPLC separation of phenolic acids and flavonoids

The developed HPLC method using binary gradient solvent systems (acetonotrile: methanol, 70:30 and glacial acetic acid: water, 0.5:99.5) and C_{18} column (250x4.6 mm internal diameter, 5µm particle size) could simultaneously separate eight phenolic acids and three flavonoids within 50 minutes at flow rate of 0.8 mL/min. The separation factors (α) of all the separated compounds were > 1.0 and the resolutions (Rs) were higher than 1.5 (data not shown). The reproducibility for separation of the phenolic acids and flavonoids was also good with RSD < 2.00% (run-to-run) and 2.70% (day-to-day) for integrated areas basis. The developed method could be used to separate phenolic acids and flavonoids in one run from samples with varied matrixes. It was used to determine the phenolic acids and flavonoids in roots, fruits and leaves extract of *T. terristris.*

The HPLC analysis of *T. terristris* phenolic acids and flavanoids profile represented in Table 2. Eight phenolic acids (Syringic acid, Gallic acid, *P*-coumaric acid, Vanillic acid, Ferulic acid, Caffeic acid and Salicylic acid) and three flavonoids (Myricetin,

Quercetin and Kaempferol) were detected and quantified. Syringic acid was found to be major phenolic acid in all the extracts except, hexane extract. Aqueous methanol extract have highest syringic acid contents (660.34 mg/ 100 g of dry plant material) followed by aqueous ethanol (655.78 mg/100g), absolute ethanol (653.33 mg/100g) and absolute methanol (594.72 mg/100g). Second major phenolic acid identified was salicylic acid.

Its quantities in aqueous methanol, aqueous ethanol, absolute methanol and absolute ethanol were found to be 640.45, 630.18, 540.92 and 626.87 mg/100g of sample, respectively. Hexane extract showed least amount of phenolic acids.

Table 2. Phenolic profile of Tribulus terristeris extracts by HPLC.

	Phenolic Content mg/100g				
Phenolic Acids	Absolute Methanol	Aqueous Methanol	Absolute Ethanol	Aqueous Ethanol	Hexane
Syringic acid	594.72±27.34	660.34±34.01	653.33±33.52	655.78±33.62	1.23±0.05
Gallic acid	120.43±6.01	137.04±6.85	132.64±5.35	135.34±5.93	0.35±0.12
P-coumaric acid	42.45±2.12	65.45±3.27	63.77±2.91	64.73±2.99	3.51±0.16
Vanillic acid	13.65±0.68	30.18±1.62	25.67±1.42	27.12±1.78	1.56±0.07
Ferulic acid	60.54±3.02	92.65±5.95	88.34±4.92	90.43±5.13	0.47±0.09
Caffeic acid	20.12±1.01	27.76±1.42	23.98±0.95	25.48±1.04	3.26±0.18
Salicylic acid	540.92±5.41	640.45±32.36	626.87±30.21	630.18±29.36	5.36±0.26
Catechin	110.65±5.53	148.34±7.41	103.12±6.14	132.13±6.27	0.26±0.01
Myricetin	385.37±20.25	423.47±21.52	371.45±18.58	399.69±21.43	0.34±0.01
Quercetin	1158.8±50.23	1345.1±60.48	1099.1±54.97	1223.1±60.32	0.61±0.02
Kaempferol	401.63±20.08	505.67±25.52	382.21±19.11	479.16±20.16	0.87±0.03

Values are mean ± standard deviation of three independent experiments (n=1×3 ×3)

Among flavonoids, quercitin was the major flavonoid detected in all the extracts. Maxomum concentration of quercetin was found in aqueous methanol extract (1345.12 mg/100g of dry plant material) followed by aqueous ethanol, absolute methanol and absolute ethanol extracts.

Antioxidant activity

Total phenolic, total flavonoids contents

The amount of total phenolics (TP) and toal flavonoids, determined from different *T. terristris* extracts were presented in Fig. 2. Major TPC was found in aqueous methnol extract (38.01 mg/g of dry sample, measured as GAE), followed by aqueous ethanol extract (32.54 mg/g), absolute methanol extract (29.42 mg/g) and absolute ethanol extract (28.34 mg/g). Hexane extract showed least TPC (3.12 mg/g). Similarly, TFC of aqueous methanol extract (11.91 mg/g of dry sample, measured as CE) was found to me the highest among all the extracts tested. TFC of aqueous ethanol, absolute methanol, absolute ethanol and hexane extracts were 10.18, 9.72, 8.54 and 1.40 mg/g of dry sample, measured as CE, respectively. Aqueous methanol extract showed significantly (P<0.05) higher TP and TF contents than others extract. TP and TF contents from extracts of varied plants were reported in literature as a marker for antioxidant potential (Hussain *et al.*, 2012; Sultana *et al.*, 2007). Many studies confirmed that amounts and composition of phenolic compounds is diversified at sub-cellular level and within the tissues (Hussain *et al.*, 2013; Sultana *et al.*, 2007). Polyphenols are a class of natural compounds that exhibited antioxidants activity and act as free radical terminators.

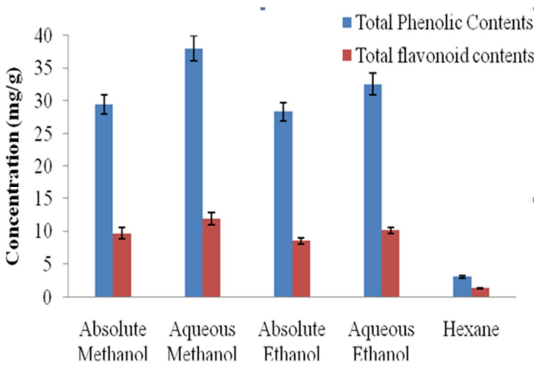


Fig. 2. Total phenolic and total flavonoid contents of *Tribulus terristeris* extracts.

DPPH radical scavenging activity

For the assessment of radical scavenging capacity of *T. terrestris* extracts, DPPH free radical scavenging assay was selected and results indicated that DPPH radical scavenging capacity of *T. terristris* extracts increased in a concentration dependent manner. The extract concentration provided 50% scavenging (IC_{50}) are given in Table 3. Aqueous methanol extracts of *T. terrestris* exhibited appreciable radical scavenging activity (IC_{50},11.35 g/mL) followed by absolute methanol (IC_{50}, 15.91 µg/ml), absolute ethanol (IC50, 16.21 µg/ml), aqueous ethanol (IC50, 11.79 µg/ml), and hexane (IC_{50}, 590.0 µg/ml). Synthetic antioxidants BHT and BHA showed better activity (IC_{50} 5.35 and 5.39 µg/ml, respectively). DPPH radical scavenging capacity of plant extract could be explained by the presence of phenolic acids and flavonoids (Siddhuraju *et al.*, 2002).

Table 3. DPPH radical scavenging capacity and antioxidant activity of *Tribulus terristeris* extracts.

Extracts	DPPH (IC_{50}) (µg/ml)	Inhibition of linoleic acid peroxidation (%)
Absolute Methanol	15.91 ± 0.51^{c}	81.21 ± 2.9
Aqueous Methanol	11.35 ± 0.43^{b}	89.40 ± 4.3^{c}
Absolute Ethanol	16.21 ± 0.39^{c}	80.50 ± 3.7^{b}
Aqueous Ethanol	11.79 ± 0.43^{b}	85.31 ± 3.9^{bc}
Hexane	590.0 ± 29.7^{d}	59.76 ± 2.6^{a}
BHA	5.35 ± 0.49^{a}	91.30 ± 3.5^{c}
BHT	5.39 ± 0.47^{a}	93.57 ± 4.1^{c}

Values are mean ± standard deviation of three independent experiments.

Values with different letters in superscript represent significant difference ($p< 0.05$) among different extracts within column.

Inhibition of linoleic acid peroxidation

The antioxidants activity of *T. terristris* extracts were also assessed by ability to prevent oxidation. Inhibition of linoleic acid peroxidation was used to assess the antioxidant activity of different *T. terristris* extracts. The aqueous methanol extract exhibited appreciable inhibition of peroxidation (89.37%) then the others solvents, that is compare able with BHA and BHT. Aqueous ethanol, Absolute methanol, absolute ethanol, and hexane extracts showed 85.31, 81.21, 80.50 and 59.76% inhibition of linoleic acid peroxidation, respectively.. These results indicate that extract from *T. terrestris* significantly reduce the formation of hydro peroxide, thus implying that this species is powerful natural antioxidants.

Reducing power

The data for the reducing potential of different *T. terristris* extracts is presented in Fig. 3. The reducing potential of the *T. terristris* extract measured for the concentration up to 10.0mg/mL, showed general increase in activity when concentration increased. As in above results, again the aqueous methanol (80% methanol) extract showed highest reducing potential than other extracts. Aqueous ethanol (80% ethanol) extract has also showed high reducing potential however, hexane extract showed poor activity. Measurement of reducing potential also reflects some aspects of antioxidant activity of plant extracts (Tanaka *et al.,* 2002). No data are available of the reducing potential of extracts with which to compare the results of our present analysis. However, Anwar *et al.* (2009) reported the good correlation index (>0.94) between concentration of fennel extract and absorbance in the reducing potential assay. Increase in the absorbance of the reaction mixture indicated increase in the reducing power. It can be observed from these results; changes on solvent polarity changes its ability to dissolve antioxidant compounds.

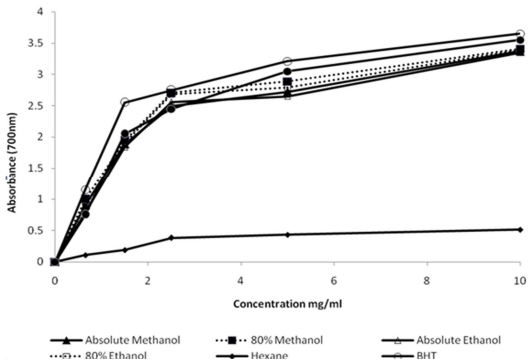


Fig. 3. Reducing potential of *Tribulus terristris* extracts.

Bleach ability of β-carotene in linoleic acid system

The antioxidant activity in terms of ability of extracts *T. terristris* to prevent the bleaching of ß-carotene was measured and presented in Fig. 4. In this study, the aqueous methanol and aqueous ethanol extracts of *T. terristris* possess good antioxidant activity than the other extracts. When compared to other solvent extracts expect BHT, BHA that is used as control shown in Fig. 4.

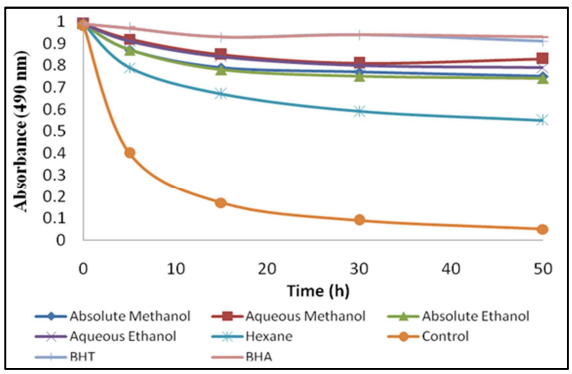


Fig. 4. Antioxidant activity of *Tribulus terristeris* extracts in term of Bleach-ability of β-carotene in linoleic acid system.

Conclusion

In conclusion, this study first time reported the phenolic profile study different *T. terristris* extracts, native to Pakistan along with antioxidant activity. Among all extracts, aqueous methanol extract of *T. terristris* contained the high TPC, TFC, and high DPPH radical scavenging capacity and antioxidant activity. *T. terristris* extracts have potential candidate for the utilization as natural antioxidants. However, further study is recommended to study the antioxidant activity of *T. terristris* extracts in food model systems.

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