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Evaluation of Free Radical Scavenging Activity and Toxic Heavy Metal Contents of Commercially Available Fruits of *Tribulus terrestris* Linn.

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Authors' contributions

This work was carried out in collaboration between both authors. Author FN designed the study, managed the analyses of the study and literature searches, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SAK also managed the literature searches and revised the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the free radical scavenging activity of organic solvent extracts and the toxic heavy metal contents of commercially available fruits sample of *Tribulus terrestris* Linn in the UAE.
Methodology: Fruit samples were extracted with hexane, ethyl acetate, ethanol and methanol solvents separately and evaluated their total polyphenol contents as well as free radical scavenging activities using 1,1-diphenyl-2-picrylhydrazyl radical assay method. For heavy metal analysis, samples were prepared by a dry ashing digestion procedure and estimated eight heavy metals: cadmium (Cd), lead (Pb), iron (Fe), arsenic (As), aluminum (Al), zinc (Zn), nickel (Ni) and copper (Cu) using Atomic Absorption Spectrometry. Method validation was performed by evaluating metal recovery studies.
Results: The free radical scavenging activities of extracts were compared with known antioxidant L-Ascorbic acid (AA) and the results were decreased in the order of: AA > methanol > ethanol >> ethyl acetate >> hexane extracts. The methanol extract was the most active free radical scavengers due to their higher polyphenols content; ethyl acetate and hexane extracts were not

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active. The fruits of *T. terrestris* exhibited a positive response for all tested heavy metals and the concentrations were recorded in 1 g sample as: 37.35±1.29 μg Al, 3.92±0.95 μg As, 0.161±0.037 μg Cd, 2.49±0.34 μg Cu, 87.93±7.87 μg Fe, 1.81±0.61 μg Pb, 0.87±0.13 μg Ni and 8.93±1.59 μg Zn. The estimated heavy metal levels were below the recommended permissible limit set by WHO except As and Fe.

Conclusion: Long time consuming of *T. terrestris* fruits might implicates an oxidative damages to cells as of their heavy metals contents and in contrary free radical scavenging activity and polyphenolic contents of extracts might give protection against this damages.

Keywords: Tribulus terrestris; Fruits; Free radicals scavenging; Heavy metals; Atomic Absorption Spectrometry.

1. INTRODUCTION

The medicinal herb *Tribulus terrestris* Linn. (Family: Zygophyllaceae) is known as Khar Khash in Arabic, Gokshura in Hindi, Gokhru in Urdu and Caltrops in English [1]. It is a prostrate annual herb found in Mediterranean, subtropical, and desert climate regions around the world [2]. It is an ingredient of Ayurvedic medicine and is used in treatment of dysuria, polyuria, oedema, bronchial asthma, piles cardiac diseases, urinary calculi, anorexia, diseases of nervous system [3]. Literature survey on phytochemical studies on fruits of *T. terrestris* showed that the herbs contained saponins [4-7], flavonoids [8,9], glycosides [10] and alkaloids [11]. Preliminary phytochemical tests for different solvent extract of fruit also showed positive response for the presence of flavonoids, alkaloids, saponins and tannins [3,12-14]. Survey on biological activity studies revealed it has diuretic activity [15,16], aphrodisiac activity [17-20], antiurolithic activity [21-23], immunomodulatory activity [24], antidiabetic activity [25-29], hypolipidemic activity [30-32], hepatoprotective activity [33], antiinflammatory activity [34,35], analgesic activity [36], antispasmodic activity [37], anticancer activity [38-41], antibacterial [42,43], anthelmintic [44] and larvicidal activity [45,46].

The use of alternative or complementary medicine has increased in developed countries [47] and within the UAE. In the UAE, the countries national citizens have a long history of using traditional herbal remedies [48] and they prefer to buy raw medicinal herbs from registered herbal shops based on their folk medicinal uses and knowledge of Ayurvedic medicine. However, the medicinal herbs always contaminated with toxic heavy metals because of contaminated soils, air and water [49]. The human influence of industrialization, pollution, waste disposal and use of fertilizer [50,51] causes soil contamination with toxic heavy metals. There are approximately 60 heavy metals known to man [52], some for example nickel and iron are essential in very low concentrations for the survival of all forms of life which are referred to as essential trace elements. Metals such as mercury, lead and cadmium are non-essential metals which are toxic if ingested in small amounts. Presence of high level of heavy metals are also reported in a number of Ayurvedic medicines and sometimes they are used as active ingredients in the formulation [47,53-57]. Therefore, there have been rising concerns over the safety of medicinal herbs due to contamination with dangerous levels of heavy metals. Patients developed symptoms of heavy metal toxicity after continuous administration of Ayurvedic medicines. In contrary, the antioxidants [58-62] may alleviate toxicity developed by heavy metals. In view of documented biological activities of fruits of *T. terrestris* and commercially availability of this herbs in many herbal shops of UAE , the present works were undertaken to evaluate the free radical scavenging activities of different organic solvents of fruits of *T. terrestris* and their correlation with their polyphenols contents as well as to evaluate trace heavy metals contents of fruits and to determine whether or not it pose a risk of heavy metal toxicity in regards to World Health Organization (WHO) levels.

2. MATERIALS AND METHODS

2.1 Plant Materials and Extraction Procedure

Authenticated dried fruits of *Tribulus terrestris* Linn. were bought from the registered herbal shop of Sharjah, UAE and a herbarium voucher specimen is deposited in the Dubai Pharmacy College for future reference. Fruits samples were further oven dried (45°C) for 24 hours, and then grounded into powder. The powdered fruits (200

g each) were extracted separately with hexane, ethyl acetate, ethanol and methanol (2 liters each) in a Soxhlet extractor for 24 hours each. A rotary vacuum evaporator was used to evaporate the solvents to dryness at 45° to 60°C. The yield of extracts was 4.17%, 4.22%, 2.05% and 1.79% for hexane, ethyl acetate, ethanol and methanol solvent extracts respectively. All the extracts were kept in refrigerator until further use.

2.2 Chemicals and Reagent

Analytical grade hexane, ethyl acetate, methanol, absolute ethanol and spectroscopic grade methanol were purchased from Merck, Darmstadt, Germany. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, Folin-Ciocalteau reagent, anhydrous sodium carbonate, were all purchased from Sigma Aldrich Chemical Co. (USA). Ammonium dihydrogen phosphate and sodium hydroxide were obtained from S D Fine-Chem Ltd (India). The hydrogen peroxide (H_2O_2) and perchloric acid ($HClO_4$) was supplied by Merck ltd (UK). The magnesium nitrate, Nickel nitrate was provided by Surechem products Ltd (UK). The hydrofluoric acid (HF) was provided by Honeywell Riedel-de Haen (Germany). The filter papers were 125 mm Qualitative circles provided by Whatman. All glassware and ceramics were Pyrex grade and polypropylene bottles were provided by Azlon (UK). All reagents were of analytical reagent grade unless otherwise stated. The element standard solutions (1 g/L) for Aluminum (Al), Arsenic (As), Cadmium (Cd), Iron (Fe), Copper (Cu), Lead (Pb), Zinc (Zn) and Nickel (Ni) were supplied by MRS Scientific Ltd, Essex (UK). Standard 1 µg/mL stock solutions of Al, As, Cd, Fe, Pb, Cu, Ni and Zn were prepared from 1 g/L standard metal solutions with 1% nitric acid. The calibration curves were constructed in the ranges as follows: Al: 0.05 – 0.3 µg/mL; As: 0.01 – 0.5 µg/mL; Cd: 0.003 – 0.05 µg/mL; Fe: 0.05 – 0.5 µg/mL; Pb: 0.001 – 0.2 µg/mL; Ni: 0.01 –0.5 µg/mL, Cu: 0.05 to 0.5 µg/mL and Zn: 0.01 – 0.5 µg/mL. Milli-Q Ultrapure (Type 1) water (Millipore, Bedford, MA, USA) was used for all dilutions of metal analysis.

2.3 Determination of Total Polyphenols Content of Extracts

The total polyphenols content of fruits extract of *T. terrestris* was determined using Folin-Ciocalteau reagent according to the procedure described by Nessa et al. [63]. The dried extracts were dissolved in methanol and sonicated. 100 µL of portion of each sample (three replicates) were transferred into the test tubes, and then added 2 mL of Folin-Ciocalteau reagent (diluted 1:10) and 2 mL of 7.5 % sodium carbonate respectively. The test tubes were then kept at room temperature (25°C) for 1.5 hr and measured the absorbance at 760 nm using Shimadzu-1700 UV-VIS spectrophotometer (Japan). Calibration curve was prepared with different concentration of quercetin solution. Total polyphenols values were expressed as milligrams of quercetin equivalent per gram of dried extract.

2.4 Determination of Free Radical Scavenging Activity

The free radical scavenging activity of four different organic solvents extracts of fruits of *T. terrestris* were evaluated using a stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH•) [63] with slight modification. Using a micropipette, 3 mL of 25 ppm methanolic solution of DPPH radical was transferred into a cuvette and then 100 µL methanolic solution of the extracts/reference standard was added and kept at 25°C for 30 minutes. Absorbance of cuvette was measured after 30 minutes at 517 nm against a blank (without antioxidant). The measurements were repeated for three times for different concentrations of antioxidants and scavenging of DPPH radicals was calculated in percent according to the formula: % Scavenging = [(AB(0) – AA(t))/AB(0)] X 100, where, AB(0) is the absorbance of the blank at t = 0 min and AA(t) is the absorbance of the antioxidant at t = 30 min. The results were expressed as SC_{50} values (concentration sufficient to obtain 50% of a maximum scavenging capacity) [63]. The dose response studies with time were also carried out and the decrease in absorbance for different concentrations of antioxidants was recorded continuously with data capturing software at 2 min intervals for 30 min with a Shimadzu-1700 UV-VIS spectrophotometer (Japan).

2.5 Instrument Parameters

The Atomic Absorption Spectrometer (AAS) AA-6800 (Shimadzu) with an auto-sampler (ASC-6100) and deuterium background correction was used for all metal analysis. The analysis of Al (309.3 nm), Cu (324.8), As (193.7 nm), Cd (228.8 nm), Ni (232.0 nm) and Pb (283.3 nm) was performed by graphite furnace. Argon gas was used for flushing the furnace. Fe (248.3 nm)

and Zn (213.9 nm) were analyzed by flame AAS using an air (oxidant) and acetylene (fuel) mixture as the flame. The air pressure used is 0.25MPa at a flow rate of 8 L/min. The standards, blank and sample solutions were analyzed for the elements of interest utilizing suitable hollow cathode lamps. The percentages of different elements in these samples were determined by the corresponding standard calibration curves. Blank experiments were carried out for all procedures.

2.6 Heavy Metal Determination

Sample was prepared by dry ashing digestion method [64] with a slight modification. About 2 g oven dried powdered samples were placed in an acid cleaned porcelain crucible with a cover and then charred at 350-400°C for 3 to 4 hours in a muffle furnace (Gallenkamp Muffle Furnace, Model: Tactical 308) until a white/grey ash was obtained. The ash was digested with conc HNO_3 (3 mL) and $HClO_4$ (1 mL) on a hotplate at 90°C for one hour. The resultant solution was diluted with 1% HNO_3, and filtered and made up to 100 mL. Magnesium nitrate and ammonium di-hydrogen phosphate were used as a matrix modifier [65] which was added to 1% HNO_3. For arsenic determination, the powdered sample was treated with an acidic mixture of HNO_3/HF (1/1) [66] in an acid cleaned Teflon screw-cap vial and the sealed containers were placed in an oven at 130°C for four hours and then allowed to cool at room temperature. The mixture was then made up to a volume of 50 mL with 1% HNO_3 and filtered. 1 mL of the aliquot from the filtrate was transferred to a 10 mL volumetric flask, and then 1mL of 1% nickel nitrate (matrix modifier) and 3% hydrogen peroxide was added and diluted to 10 mL with Milli-Q water. The metals were quantified against 5-point calibration curves. Analytical recovery was determined by adding different measured amounts (in μg) of three selected heavy metals (As, Ni and Pb) to powdered sample. A control sample was prepared without adding heavy metals and all the samples were prepared using the methods of sample preparation and analyzed by AAS. The recovery data was determined by subtracting the values obtained from control and added standard samples. This procedure was repeated three times and a standard deviation was produced.

2.7 Statistical Analysis

The data results were compared for each extract and subjected to a one-way analysis of variance (ANOVA). Tukey's test (P = .05) was performed to determine the significance of the difference between means.

3. RESULTS AND DISCUSSION

3.1 Total Extractive Values and Polyphenol Contents of Different Solvent Extracts

Total extractive values of different solvent extracts of *T. terrestris* were recorded and the % yields were decreased in the order of: hexane (4.17) > ethyl acetate (4.22) > ethanol (2.05) > methanol (1.79). It seemed that fruits samples contained more non-polar compounds than polar compounds. The total polyphenol contents of different solvent extracts of fruits of *T. terrestris* were also determined and the results were expressed as milligrams of quercetin equivalent per gram of dried extract. Here, quercetin is used as a reference compound for polyphenols content quantitation as it is a naturally occurring potent bioactive flavonoid and it has metal chelating activity as well as protective activity against oxidative damages of cells by arsenic, cadmium, copper and iron [62,67-71]. The results showed that methanol extracts have the highest polyphenolic content (mg/g of dried extract) whereas hexane has the least content of polyphenols. The total polyphenols content of each extract as assessed was decreased in the order of: methanol (29.12±1.16) > ethanol (27.28±1.88) > ethyl acetate (2.87±1.01) > hexane (0.56±0.05). There was no statistically significant difference (P = .05) in polyphenol contents between the methanol and ethanol extracts. The results are shown in Table 1.

3.2 The Free Radical Scavenging Activities of Different Solvent Extracts of *T. terrestris*

The free radical scavenging activities of different solvent extracts of *T. terrestris* were determined using the DPPH assay as shown in Fig. 1. The results were expressed as SC_{50} values, were calculated by regression analysis and are presented in Table 1. The result was decreased in the order of: L-Ascorbic acid > methanol extract > ethanol extract >> ethyl acetate extract >> hexane extract. Hexane and ethyl acetate extract was inactive towards the working concentration (100 to 500 μg/mL). L-Ascorbic acid is a known antioxidant and was used in this experiment as a reference compound for

comparison. Methanol and ethanol extracts showed significantly lower SC_{50} values (P = .05) than L-Ascorbic acid. The marked antioxidant activity of methanol/ethanol extract attributed to their polyphenols contents. Polyphenols are believed to intercept the free radical chain of lipid oxidation and to donate hydrogen from the phenolic hydroxyl groups, thereby forming a stable end product, which does not initiate or propagate further oxidation of the lipid [72]. Hence methanol and ethanol extracts merits to use in biological system as cytoprotective in radical mediated diseases.

The dose-response studies of DPPH scavenging reaction of extract at various concentrations in 30 minutes reaction showed that the steady state of the reaction was not achieved at 30 minutes. All the extracts showed steady state reaction at high concentrations. The extracts of *T. terrestris* reacted slowly with DPPH radical and scavenging activity increased with increasing

Table 1. Percent yields, total polyphenols content and SC_{50} values of different solvent extracts of the fruits of *T. terrestris* for scavenging of free radicals as assessed with DPPH radical scavenging method

Solvent extracts of fruits of *Tribulus terrestris*	% Yields	Total polyphenols content (mg/g of dried extract±S.D.)	*SC_{50} (µg/mL)±S.D.	*r
Methanol extract	1.79	29.12±1.16	219.76±2.12	0.98
Ethanol extract	2.05	27.28±1.88	285.98±1.56	0.98
Ethyl acetate extract	4.22	2.87±1.01	Not active at working concentrations	-----
Hexane extract	4.17	0.56±0.05	Not active at working concentrations	-----
L-Ascorbic acid	-----	-----	86.2 ± 1.72	0.95

**The concentration sufficient to obtain 50% of a maximum scavenging capacity. SC_{50} values were calculated from linear regression lines where: r = correlation coefficient. Results are mean±S.D (n = 3).*

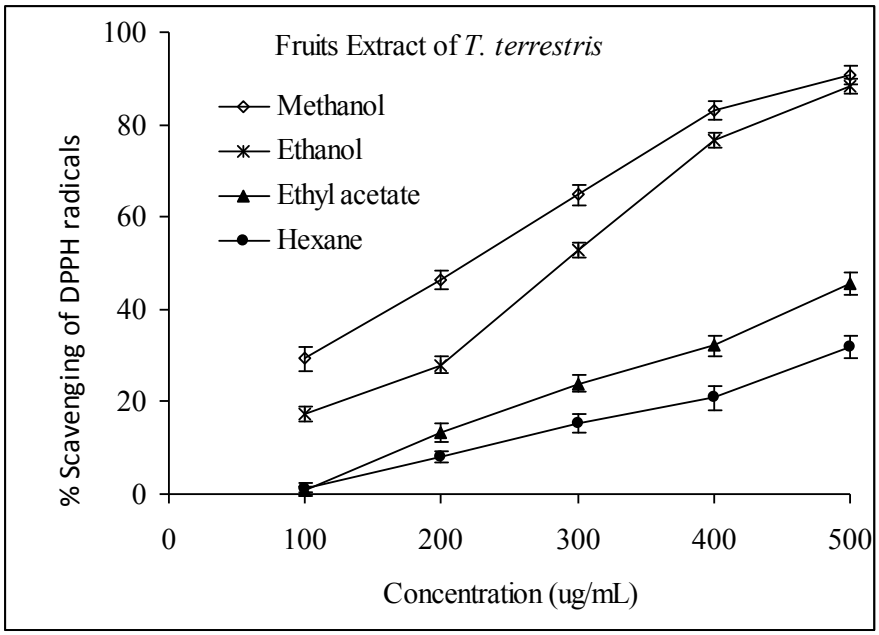

Fig. 1. Free radical scavenging activity of different solvent extracts of *Tribulus terrestris* fruits measured using the DPPH-assay. Results are mean±S.D (n = 3)

concentration from 100 to 500 µg/mL. The ethyl acetate and hexane extract were very poor free radical scavengers and slowly scavenged free radicals. However, methanol extracts exhibited comparatively steady state reaction at high concentrations. The data of dose-response studies of DPPH scavenging reaction of *T. terrestris* are presented in Figs. 2-5.

3.3 Heavy Metal Contents of *T. terrestris* Fruits

The calibration curves for the heavy metals were linear over the different concentration ranges and the regression coefficients (r) were 0.9985 (Al), 0.9979 (Cd), 0.9989 (As), 0.9979 (Fe), 0.9996 (Cu), 0.9973 (Ni) 0.9977 (Pb), and 0.9981 (Zn)

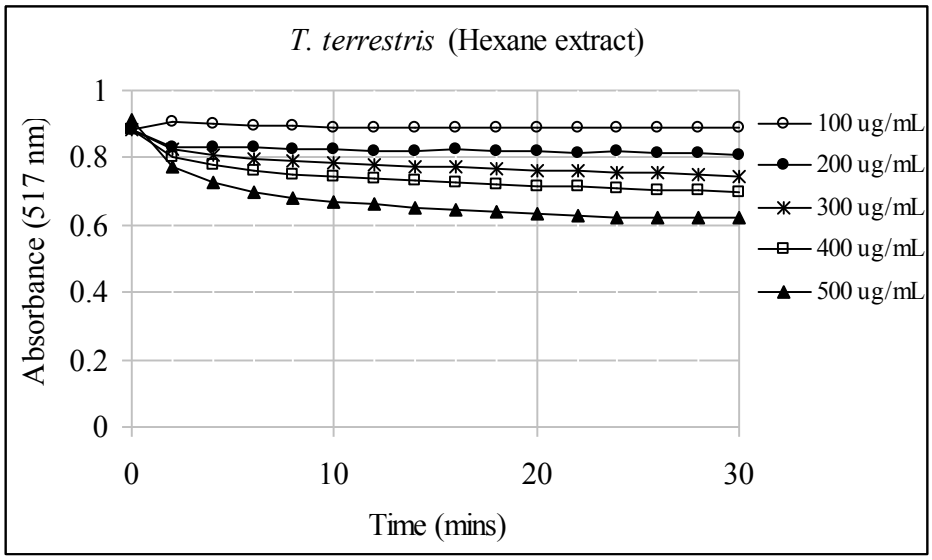

Fig. 2. Hydrogen donating abilities of different concentration of hexane extract of *Tribulus terrestris* on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

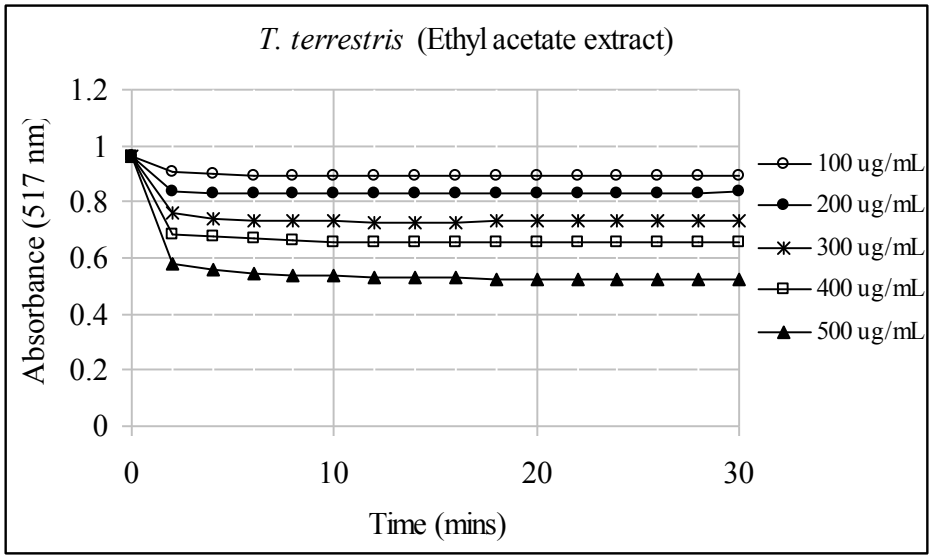

Fig. 3. Hydrogen donating abilities of different concentration of ethyl acetate extract of *Tribulus terrestris* on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

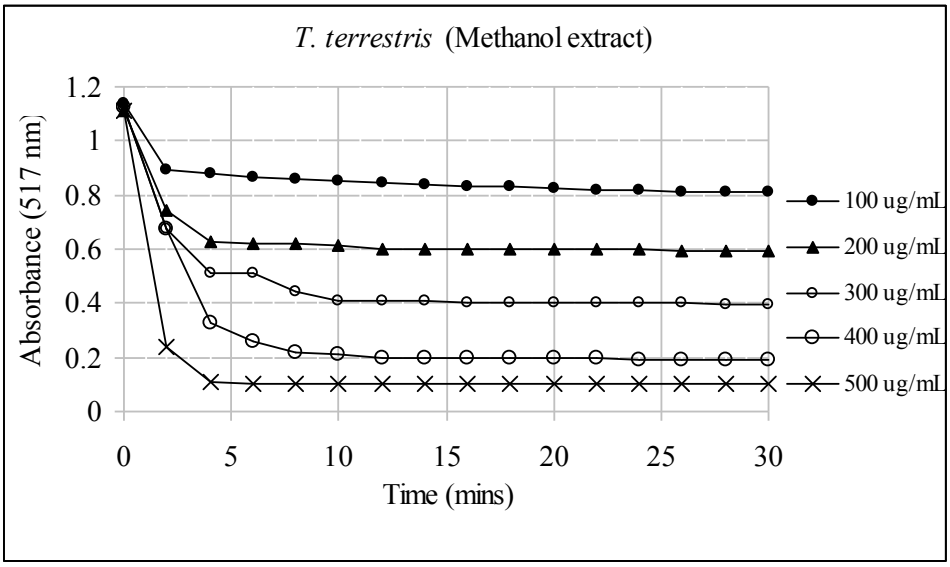

Fig. 4. Hydrogen donating abilities of different concentration of methanol extract of *Tribulus terrestris* on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

respectively. The minimum detection limits were 1 ng/mL for Al, As, Cd, Ni and Pb respectively; whereas for Fe and Zn was 100 ng/mL and for Cd was 0.25 ng/mL. The mean analytical recoveries were in the ranges of 88.40% to 97.30%, which confirms the suitability of the sample preparation procedure for quantifying heavy metals. The values of the mean recovery studies are illustrated in Table 2. Three fruits samples of *T. terrestris* were assayed for eight heavy metals and the results were expressed as µg/g of dried samples and shown in Table 3.

3.3.1 Iron (Fe)

The three fruits samples of medicinal herb *T. terrestris* exhibited positive response for Fe. The level of iron recorded in the ranges of 81.22 to 96.11 µg/g. Statistical analysis within groups showed that there were significant differences (P = .05) of Fe content in between sample 2 and 3 and no significant differences with sample 1. The WHO recommended level of Fe in medicinal plants is 20 mg/kg, while its dietary intake is 10–28 mg/day [73]. After comparison, with the proposed limit of Fe it was found that all the samples exhibited higher Fe content than the established limit. Though Fe is an essential element in our body, however, Fe overload causes a number of toxic effects such as enlarged liver, skin pigmentation, lethargy, joint diseases, and loss of body hair, amenorrhea, and impotence [74-76].

3.3.2 Aluminum (Al)

The level of Al recorded in fruits samples are in the ranges of 36.91 to 38.11 µg/g. Statistically, there were no significant differences (P = .05) in Al contents amongst the sample 1, 2 and 3. The WHO recommended level of Al in medicinal plants not yet established. However, WHO recommended the tolerable limit for aluminum from finished herbal products is 1 mg/kg body weight (BW) per week [77,78]. According to WHO, an adult of body weight 70 kg can tolerate almost 10 mg aluminum per day. Although Al is not considered as heavy metal, environmental exposure is frequent from foods, cookware, soda cans and treated drinking water [79]. Chronic exposure to Al increases the immunoreactivity within the hippocampus and slows down reaction time and is linked to Alzheimer's disease [80-82].

3.3.3 Copper (Cu)

The recorded level of Cu in fruits samples were 2.44 to 2.55 µg/g. There were no statistical significant differences (P = .05) in Cu contents amongst the studied three samples (1, 2 and 3). The WHO recommended level of Cu in medicinal plants is 10 mg/kg [83] and all the samples showed lower levels of Cu than WHO recommended level. Although Cu plays an important role in human nutrition, but elevated

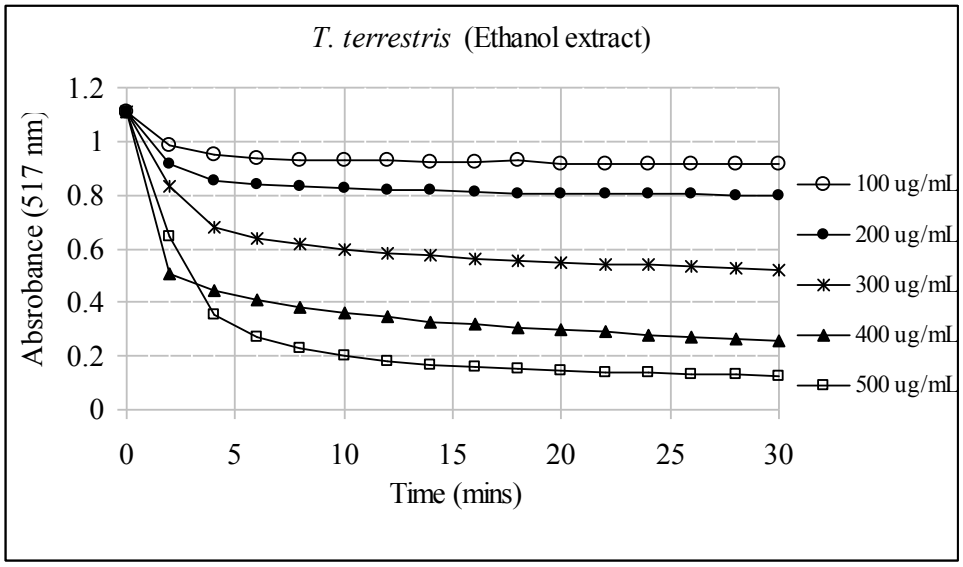

Fig. 5. Hydrogen donating abilities of different concentration of ethanol extract of *Tribulus terrestris* on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

Table 2. Mean recoveries of heavy metals from *T. terrestris* fruits powder

Metal	Amount added to fruits powder (µg)	Amount recovered (µg)*	Mean Recovery (%)
Pb	0.25	0.221±0.005	88.40
	0.50	0.483±0.014	96.60
	1.00	0.973±0.011	97.30
Ni	0.25	0.235±0.015	94.00
	0.50	0.475±0.024	95.00
	1.00	0.971±0.051	97.10
As	0.25	0.218±0.065	87.20
	0.50	0.477±0.034	95.40
	1.00	0.969±0.055	96.90

** Results are expressed as Mean±standard deviation (n = 3)*

Table 3. Heavy metal contents of fruits of *T. terrestris* determined by Atomic Absorption Spectrometry. Results are mean±S.D (*n* = 3)

Fruits of *Tribulus terrestris*	Fe (µg/g)	Cu (µg/g)	Al (µg/g)	Ni (µg/g)	As (µg/g)	Pb (µg/g)	Zn (µg/g)	Cd (µg/g)
Sample # 1	86.45±6.78	2.49±0.29	37.03±1.25	0.78±0.04	4.57±0.65	1.46±0.71	8.61±1.21	0.133±0.01
Sample # 2	96.11±4.18	2.55±0.52	36.91±1.41	1.01±0.12	3.11±0.88	2.06±0.45	9.01±2.11	0.201±0.01
Sample # 3	81.22±3.55	2.44±0.32	38.11±1.35	0.82±0.09	4.09±0.87	1.91±0.69	9.17±1.99	0.149±0.004
Total	87.93±7.87	2.49±0.34	37.35±1.29	0.87±0.13	3.92±0.95	1.81±0.61	8.93±1.59	0.161±0.037

exposure to Cu leads to neurotoxicity [84,85], hair and skin decolouration, dermatitis, metallic taste in the mouth and nausea [86].

3.3.4 Nickel (Ni)

The recorded level of Ni in the fruits sample of *T. terrestris* were 0.78 to 1.01 µg/g, however, there were no significant differences (*P* = .05) in Ni contents amongst the sample 1, 2 and 3. The WHO permissible limit of Ni in medicinal plants is 1.5 mg/kg [83] and all the samples showed lower levels of Ni than WHO recommended level. The nickel toxicity in human is not very common occurrence because it is poorly absorbed in the body [82]. Ni is required in minute quantity for body, however, over exposure of Ni leads to allergic dermatitis, encephalopathy, and reduced

sperm count and also adversely affects lungs and nasal cavities [87,88].

3.3.5 Arsenic (As)

Studied three fruits samples of *T. terrestris* exhibited positive response for As. The ranges of As were 3.11 to 4.57 μg/g and there were no statistical significant differences (*P* = .05) in As contents amongst the tested samples. The WHO recommended level of As in medicinal plants was established as 1 mg/kg [89]. According to WHO, the permissible level of arsenic intake per weakly is 15μg/kg BW [90]. However, the recorded arsenic level was higher than WHO permitted level. As is one of the more commonly reported hazardous element [85] and chronic exposure to As causes Nausea, vomiting, diarrhoea, painful neuropathy, diabetes, cancer [88,91]. Other symptoms are anorexia, fever, mucosal irritation, skin lesions on the palms and soles of the feet and arrhythmia. Cardiovascular changes are often subtle in the early stages, but can progress to cardiovascular collapse [92-94].

3.3.6 Lead (Pb)

The recorded level of Pb in fruits samples were in the ranges of 1.46 to 2.06 μg/g. There were no statistically significant differences (*P* = .05) in Pb contents amongst the three samples. The WHO recommended level of Pb in medicinal plants is 10 mg/kg [73,89] and the results obtained from our study showed lower level of Pb contents than WHO recommended level. Pb has no known beneficial function in human metabolism [80]. However, with repeated consuming this medicinal herbs might cause lead poisoning. It is well documented that chronic exposure to lead may result in a number of toxic effects as birth defects, mental retardation, autism, psychosis, allergies, dyslexia, hyperactivity, weight loss, shaky hands, muscular weakness, and paralysis [85]. In addition symptoms of chronic lead exposure causes allergies, arthritis, autism, colic, hyperactivity, mood swings, nausea, numbness, lack of concentration, seizures and weight loss [82,88,95].

3.3.7 Zinc (Zn)

The level of recorded Zn in the studied three fruits samples of *T. terretris* were in the ranges of 8.61 to 9.17 μg/g. There were no statistically significant differences (*P* = .05) in Zn contents amongst the studied samples. The permissible limit set by WHO [73] in medicinal herbs is 50 mg/kg. All the fruits samples exhibited lower level of zinc than WHO recommended level. Zinc is an essential trace element and plays an important role in various cell processes including normal growth, brain development, behavioral response, bone formation and wound healing. However, chronic exposure of Zn causes vomiting, diarrhoea, abdominal pain, anaemia, neurological degeneration and osteoporosis [88].

3.3.8 Cadmium (Cd)

The tested three samples of fruits of *T. terretris* exhibited positive response for Cd. The highest level recorded in sample 2 (0.201μg/g) and lowest level in sample 1 (0.133 μg/g). The mean differences of sample 1 and 2 were significantly different at the level of .05, however, the mean differences between sample 1 and 3 were insignificant (*P* = .05). The recommended level for Cd in medicinal herbs was 0.3 mg/kg as per reported by WHO [83,89]. In comparison with WHO limit, our studied results exhibited lower level of Cd. Cadmium has no known beneficial role in human metabolism and acute Cd poisoning is very rare [85]. Following chronic exposure of Cd causes a number of toxic effects such as obstructive lung disease, kidney disease, and fragile bones, alopecia, anemia, arthritis, learning disorders, migraines, growth impairment, emphysema, osteoporosis, loss of taste and smell, poor appetite and cardiovascular disease [82,88,96,97].

4. CONCLUSION

The free radical scavenging activity of four different solvent extracts of fruits of *T. terrestris* revealed that methanol extracts was highly potent against scavenging of free radicals than ethanol extract. The SC_{50} values of extracts were compared with natural antioxidant L-Ascorbic Acid (AA) and AA exhibited significantly (*P* = .05) higher activity against scavenging of free radicals than fruits extracts. L-Ascorbic acid is a natural antioxidant and free radical scavengers and can protect cells from oxidative damages produced by heavy metals such as lead [58], mercury [59], arsenic and cadmium [60]. The present study revealed the presence of trace levels of toxic heavy metals such as Al, Fe, Cd, Ni, Zi, As, Pb and Cu in the commercial samples of *T. terrestris* fruits. Though the recorded levels of heavy metals were not in the alarming ranges, however, long term consuming of this fruits samples may implicates heavy metal toxicity. In addition, the antioxidant activities of methanol and ethanol extracts of *T. terrestris* fruits can protect oxidative

damages caused by heavy metals due to their quercetin equivalent polyphenol contents.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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