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Evaluation of *in vitro* free Radical Scavenging Activity of *Tribulus terrestris*

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Abstract—*Tribulus terrestris L. (Zygophylaceae) is an annual medicinal plant. The present study was assessed to determine the antioxidant activity of various extracts by using DPPH (1, 1-diphenyl-2-picryl hydrazyl radical) radical scavenging activity and superoxide radical scavenging activity. The five different solvent extracts (petroleum ether, chloroform, acetone, methanol and water) were prepared from leaves by soxhlet extraction method in ascending order of polarity. The % yield of extracts obtained was 5.12 %, 28.94 %, 15.43 %, 22.32 % and 34.75 % in petroleum ether, chloroform, acetone, methanol and water respectively. Phytochemical analysis of various extracts revealed the presence of many phytoconstituents including tannins, glycosides steroids, terpenoids, flavonoids, alkaloids etc. Presence of phenolic compounds in leaves extract of T. terrestris indicates an antioxidant potential. According to DPPH radical scavenging activity, chloroform extract exhibited maximum inhibition with IC50 value i.e. 81.85µg/ml. The superoxide radical scavenging activity of various extracts evaluated that petroleum ether extract and water extract exhibited maximum scavenging with IC50 value i.e. 3.69µg/ml and 13.52µg/ml respectively. Results of the present study indicate that T. terrestris can be exploited for protection against oxidation damage by free radicals.*

1. INTRODUCTION

Free radicals are reactive nitrogen and oxygen species which are produced by a variety of metabolic processes in the body. Uncontrolled generation of free radicals leads to dysfunction in membrane lipids, proteins, enzymes and DNA causing oxidative stress and cell death. These are responsible for many degenerative human diseases like diabetes mellitus, cancer, neurodegenerative disorders, Alzheimer's disease, Parkinson's disease, atherosclerosis, ageing and other inflammatory diseases [1]. In human body, there are several free radical scavenging systems but micronutrients like vitamin E, beta-carotene and vitamin C are the major antioxidants. These must be provided in diet because body cannot produce these nutrients [2]. Protection against free radicals can be enhanced by taking adequate amounts of exogenous antioxidants. An antioxidant is a steady molecule which donates an electron to a rampaging free radical and terminates the reaction before vital macromolecules are damaged. This property of antioxidants delays or inhibits cellular damage [3]. Natural phenolic compounds play an important role in cancer prevention and treatment. Phenolic compounds from medicinal herbs include phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, and others [4]. Several activities of phenolic compounds are responsible for their chemopreventive properties (e.g., antioxidant, anticarcinogenic, or antimutagenic and anti-inflammatory effects) and also contribute to their inducing apoptosis by arresting cell cycle, regulating carcinogen metabolism and ontogenesis expression [5]. A variety of medicinal plants and their purified phytoconstituents have shown therapeutic potentials. Various herbs and spices have been reported to exhibit antioxidant activity such as *Ocimum sanctum*, *Piper cubeba* Linn., *Allium sativum* Linn., *Terminalia bellerica*, *Camellia sinensis* Linn., *Zingiber officinale* and several other plants [6]. *In vitro* experiments on antioxidant compounds from higher plants showed that they protect against oxidation damage by inhibiting or quenching free radicals and reactive oxygen species [7].

Tribulus terrestris Linn. is an annual herb, belongs to the family Zygophyllaceae. It is commonly known as puncturevine, caltrop, goat head, bull's head, ground burr nut and devil's thorn. It generally grows up to 10–60 cm. Stems are simple or freely branched. Leaves are opposite, often unequal and paripinnate. Flowers are 4–5 mm in size with yellow petals. Fruit are stellately arranged, hard with rugose carpels which are keeled, tuberculate on the back and with 2 or more stout spines on the sides [8]. It is usually found in waste places and dry habitats throughout the warmer regions of India and other regions of world. The various plant parts are known to be used as traditional herbal medicine to treat various ailments such as kidney infection, impotence and cancer etc. Fruits of this herb have antihypertensive activity [9]. Considering the traditional potentiality of *T. terrestris* as an anticancer herb, a preliminary investigation was undertaken to examine its phytoconstituents and *in vitro* antioxidant potential.

2. MATERIALS AND METHODS

2.1. Plant Material

Fresh leaves of *T. terrestris* were collected from campus of Maharshi Dayanand University, Rohtak, Haryana, India, in June 2012. The plant was further identified from Department

of Botany, Maharshi Dayanand University, Rohtak, Haryana (India).

2.2. Chemicals and reagents

Petroleum ether, chloroform, acetone, methanol, 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH), ascorbic acid, nitro blue tetrazolium (NBT), phosphate buffer, NADH, phenazine metho-sulphate (PMS) and gallic acid were purchased from Hi-Media.

2.3. Sample preparation and extraction

The plant materials were shade dried for three weeks. The dried plant materials were grinded to powdered state. The powder was weighed before extract preparation. Then, the powdered plant materials were extracted successively with different solvents i.e. petroleum ether, chloroform, acetone, methanol and water [10]. The most common and popular method is the Soxhlet's extraction. In this method, dried plant materials were extracted separately in different solvents according to increasing polarity i.e. petroleum ether, chloroform, acetone, methanol and water. The extract was filtered and the filtrate was evaporated under reduced pressure to obtain crude extract of the plant. The percentage yields of crude extracts in different solvents were calculated by the following formulae:

Percentage yield =

$$\frac{\text{Weight of crude extract obtained in grams}}{\text{Total weight of dried plant material in grams}} \times 100$$

2.4. Phytochemical screening

The phytochemical analysis of five different solvent extracts was performed by following the classical methods described by Harbone [11].

2.5. Antioxidant Assays

2.5.1. DPPH radical scavenging activity. In DPPH radical scavenging activity, samples (1mg/ml) were prepared by dissolving dry extracts in methanol. An aliquot of 2 ml of 0.004% DPPH solution in methanol was mixed with 1 ml different extracts in methanol at various concentrations (1000, 500, 250, 125, 62.5 and 31.25 µg/ml) and incubated at 25°C for 30 min. Absorbance of the test mixture was read at 517 nm using a spectrophotometer (Shimadzu) against a DPPH control containing only 1 ml of methanol. All experiments were performed along with ascorbic acid (standard) [12]. Percent inhibition was calculated using the following formulae;

% Inhibition = (Ablank – Asample /Ablank) x 100

Where "Ablank" and "Asample" stand for absorption of the blank and test sample respectively.

2.5.2. Superoxide radical scavenging activity. Nitro blue tetrazolium (NBT) reduction method was used to determine superoxide radical scavenging activity. The reaction mixture was prepared by mixing 1.0 ml of NBT solution (312 µM NBT in 100 mM phosphate buffer, pH 7.4), 1.0 ml NADH solution (936 µM NADH in 100 mM phosphate buffer, pH 7.4) and 0.1 ml different extracts of *T. terrestris* at different concentrations (1000, 500, 250, 125, 62.5 and 31.25 µg/ml). Further 100 µL of phenazine methosulphate solution (120 µM PMS in 100 mM phosphate buffer, pH 7.4) was added to the mixture. The tubes were incubated for 15 minutes and the optical density was measured at 560 nm using spectrophotometer (Shimadzu). Gallic acid was used as standard [13]. The percentage scavenging was calculated by following formulae:

% scavenging = (1- Ae / Ao) × 100.

Where "Ao" is the absorbance of mixture without sample and "Ae" is the observance of mixture with the sample.

2.5.3. Calculation of inhibition concentration (IC50). The IC50 value is defined as the amount of antioxidant necessary to decrease the initial concentration of the free radical activity by 50%. IC50 values were calculated from the graph plotted between % scavenging activities against the concentrations of the samples.

3. RESULTS

Various extracts of *T. terrestris* (leaves) screened for antioxidant activity. The percentage yield of five extracts and phytochemicals present in different extracts are shown in Fig. 1 and Table-1 respectively.

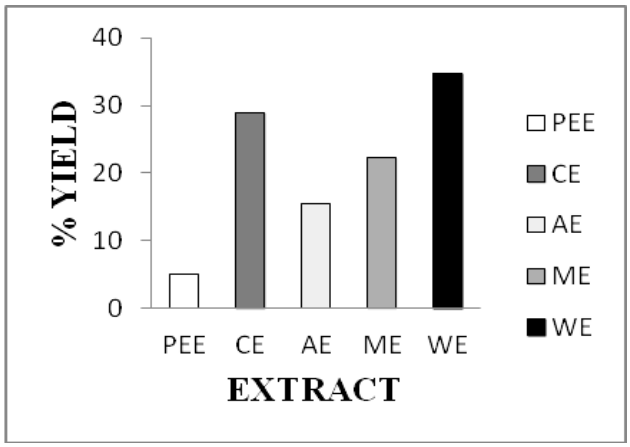

Fig. 1: Percentage yield of different plant extracts. (PEE- Petroleum ether extract, CE- Chloroform extract, AE- Acetone extract, ME- Methanol extract, WE- Water extract).

Table 1: Various phytochemicals present in different extracts of *T. terrestris* (leaves).

Sr. No.	Extracts	Phytochemicals						
		A	T	S	G	SA	F	TE
1.	PEE	+	+	+	-	+	+	+

2.	CE	+	+	+	+	+	+	-
3.	AE	-	+	-	-	-	+	+
4.	ME	-	-	+	-	+	+	-
5.	WE	+	+	+	-	+	+	+

*A- Alkaloids, T- Tannins, S- Steroids, G-Glycosides, SA- Saponins, F- Flavonoids, TE- Terpenoids, PEE- Petroleum ether extract, CE- Chloroform extract, AE- Acetone extract, ME- Methanol extract, WE- Water extract, - indicates absence and + indicates presence of phytochemicals.

3.2. Antioxidant assays

Different extracts of *T. terrestris* (leaves) evaluated for their antioxidant activity by DPPH radical scavenging assay and superoxide radical scavenging assay.

3.2.1. DPPH radical scavenging assay. The DPPH radical scavenging activities of five extracts of *T. terrestris* (leaves) are shown in Figure 2 and 3. Chloroform extract exhibited maximum inhibition in comparison with ascorbic acid (standard) with IC50 values i.e. 81.85µg/ml and 15.55µg/ml respectively. However, petroleum ether extract showed minimum inhibition with IC50 value i.e. 743.02µg/ml. IC50 values of different extracts along with ascorbic acid (standard) are given in (Table 2).

3.2.2. Superoxide radical scavenging assay. According to superoxide radical scavenging assay, the petroleum ether extract exhibited maximum radical scavenging in comparison with gallic acid (standard) with IC50 values i.e. 3.69µg/ml and 4.97µg/ml. Water extract showed radical scavenging activity with IC50 value of 13.52µg/ml while methanol extract found to possess least radical scavenging activity with IC50 value of 765.59µg/ml µg/ml. IC50 values of different extracts along with gallic (standard) are given in (Table 2). The superoxide radical scavenging activities of five extracts of *T. terrestris* (leaves) are shown in Figure 4 and 5.

Table 2: IC50 values of different extracts from DPPH radical scavenging assay and superoxide radical scavenging assay along with ascorbic acid (standard) and gallic acid (standard) respectively

Sr. No.	Plant Extracts	DPPH radical scavenging assay IC50 (µg/ml)	Superoxide radical scavenging assay IC50 (µg/ml)
1.	Petroleum ether	743.02	3.69
2.	Chloroform	81.85	154.17
3.	Acetone	467.74	463.45
4.	Methanol	292.42	765.59
5.	Water	407.38	13.52
6.	Ascorbic Acid	15.55	-
7.	Gallic Acid	-	4.97

- indicates absence of standard used in particular antioxidant assay.

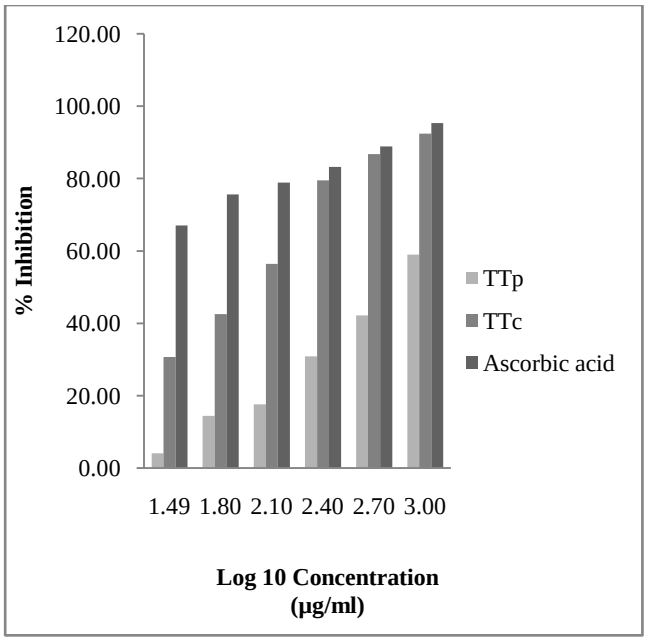

Fig. 2: DPPH Scavenging activity of *T. terrestris* (leaves) extracts. TTp and TTc indicates petroleum ether extract and chloroform extract of *T. terrestris* (leaves) respectively.

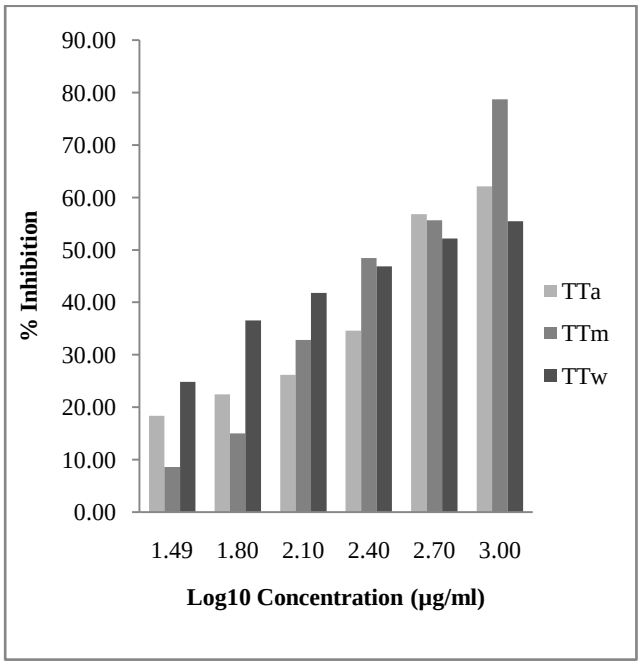

Fig. 3: DPPH Scavenging activity of *T. terrestris* (leaves) extracts. TTa, TTm and TTw indicates acetone extract, methanol extract and water extract of *T. terrestris* (leaves) respectively.

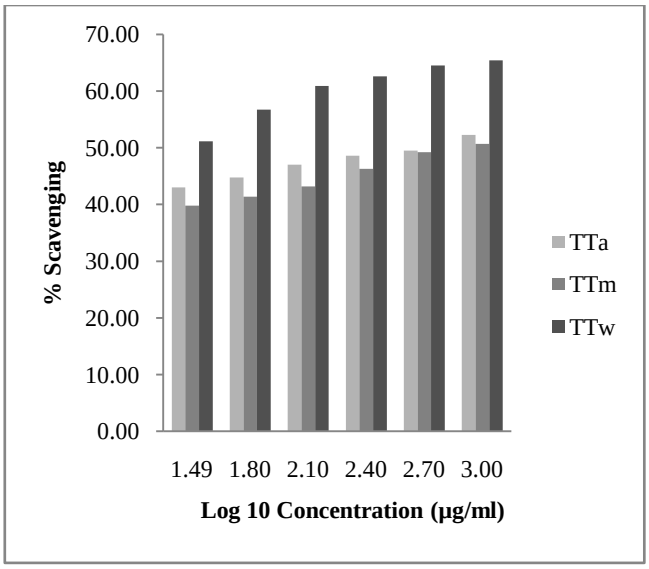

Fig. 4: Superoxide radical scavenging activity of *T. terrestris* (leaves) extracts. TTa, TTm and TTw indicates acetone extract, methanol extract and water extract of *T. terrestris* (leaves) respectively.

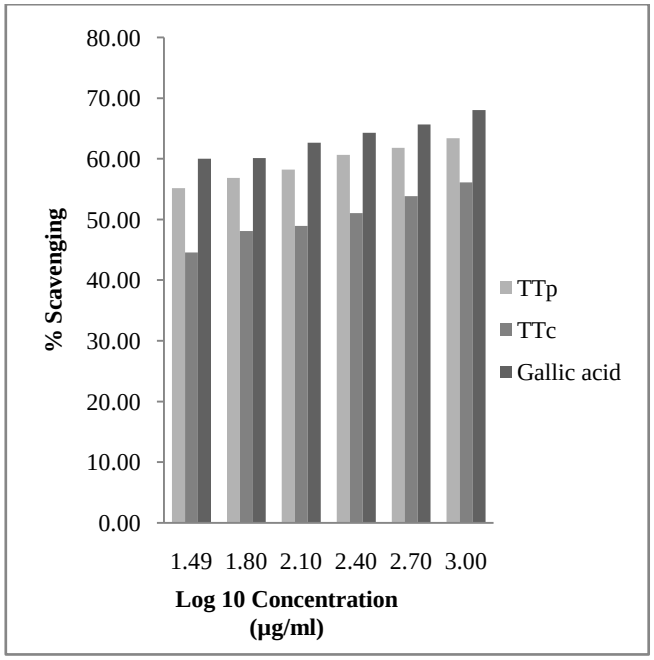

Fig. 5: Superoxide radical scavenging activity of *T. terrestris* (leaves) extracts. TTp and TTc indicates petroleum ether extract and chloroform extract of *T. terrestris* (leaves) respectively.

4. DISCUSSION

Reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide play a critical role in the development of several diseases such as arthritis, dementia, carcinoma and many other diseases due to oxidative damage [5]. Many of the drugs isolated and characterized from plants have a folklore origin and are traditionally employed in human medicine systems [9]. Natural antioxidants that are present in herbs are responsible for inhibiting or preventing the harmful consequences of oxidative stress. Medicinal herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds [14]. Numerous studies revealed DPPH radical scavenging activity of extracts from different medicinal plants such as *Acacia catechu*, *Citrus limon* 22, *Stevia rebaudiana* etc [15]. In the present study, the leaves extracts of *T. terrestris* in five different solvents has been evaluated for its free radical scavenging activity. In this study, preliminary phytochemical investigation of five extracts of *T. terrestris* (leaves) confirms the presence of alkaloids, phenols, flavonoids, saponins, glycosides and terpenes. Phenols and terpenes are the main chemical constituents responsible for free radical scavenging activity and hence act as primary and secondary antioxidants [16, 17]. Similar studies on free radical scavenging activity of *T. terrestris* have been performed previously. Antioxidant potential of *T. terrestris* L. herbal preparations was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt (ABTS) free radicals, ferric reducing antioxidant power (FRAP) and inhibition of lipid peroxidation by ferric thiocyanate method (FTC) [15]. Methanol extract of *T. terrestris* has reported with free radical scavenging activity in DPPH radical scavenging assay and super oxide radical scavenging assay with IC50 values i.e. 0.65 mg/ml and 0.70 mg/ml respectively [18]. In the present study, chloroform extract showed maximum scavenging activity in DPPH radical scavenging assay. For superoxide radical scavenging assay, maximum activity exhibited in the petroleum ether extract. Hence, the present investigation clearly indicated the antioxidant potential of *T. terrestris.*

5. CONCLUSION

Antioxidant-rich plant extracts serve as sources of nutraceuticals that ease the oxidative stress and therefore prevent the degenerative diseases. An attempt has been made to explore the antioxidant properties of *T. terrestris* (leaves) extracts. The present investigation suggests that medicinal plants which possess good antioxidant potential are the best supplements for the diseases associated with oxidative stress. Furthermore, *in vivo* studies can also explore the mechanism of antioxidant action.

6. ACKNOWLEDGEMENTS

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