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Research Article

Evaluation of antioxidant potential of Indian wild leafy vegetable *Tribulus terrestris* .

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ABSTRACT

Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against various infections and degenerative diseases. Polyphenols have been proved to possess antioxidant activities and thus have been used for the prevention and cure of diseases which are mainly associated with free radicals. The present investigation was undertaken to screen the phytochemicals, *in vitro* antioxidant and assessment of free radical scavenging activity of aqueous and alcoholic extracts of *Tribulus terrestris* Linn. (Zygophyllaceae) by using different analytical methods viz, 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay, hydrogen peroxide radical scavenging assay, polyphenol contents, etc. Aqueous and alcoholic extracts of *T. terrestris* exhibited concentration dependent free radical scavenging activity in all models studied. Total phenolic content of alcoholic extract of *T. terrestris* is higher than corresponding aqueous extract.

Keywords: *Tribulus terrestris,* DPPH radical assay, hydrogen peroxide scavenging assay, total phenolic content

INTRODUCTION

T. terrestris linn.(Zygophyllaceae) is an annual herb, commonly found throughout India up to an altitude of 5400 meters. It mostly grows wild especially in West Rajasthan, Gujarat, Maharashtra, UttarPradesh and also in other states of India. It is popularly known as 'puncture vine' and shows worldwide distribution[1]. Since ancient times it is regarded as an aphrodisiac in addition to its beneficial claims on various ailments such as urinary infections, inflammations, oedema and ascites[2]. The extract of drug is also prescribed in nephritis and kidney stones for painful micturation and for treatment of gout as well. *T.terrestris* is a common ingredient of an Ayurvedic preparations "dashmoolarishta and chyavanprash"[3].

Majority of diseases or disorders are mainly linked to oxidative stress due to free radicals[4, 5]. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism[4, 6]. The oxidative stress depicts the existence of products called Free radicals and Reactive Oxygen Species (ROS), which are formed under normal physiological conditions but become more harmful and deleterious when not eliminated by the endogenous systems. In fact, oxidative stress results from an imbalance between the generation of ROS and endogenous antioxidant systems[6, 7, 8.]

The most common ROS include superoxide (O_2^-) anion, hydrogen peroxide (H_2O_2), peroxyl (ROO^-) radicals and reactive hydroxyl (OH^-) radicals. The nitrogen derived free radicals are nitric oxide (NO^-) and peroxynitrite anion ($ONOO^-$) radicals[4, 9]. ROS are major sources of primary catalysis that initiate oxidation *in vivo* and *in vitro* and create oxidative stress which results in numerous diseases and disorders such as cancer, cardiovascular disease, neuronal disorders, Alzheimer's diseases, mild cognitive impairment, Parkinson's diseases, alcohol induced liver disease, ulcerative colitis, atherosclerosis, etc. Reactive Oxygen Species are also proved to be cytotoxic and cause tissue injuries[4, 9, 10.]

In treatment of such diseases, antioxidant therapy has gained an immense importance. Antioxidants are

able to give electrons to the free radicals and thus eliminating the adverse effects by stabilizing them[4]. Phytochemicals might exert antioxidant action *in vivo* or in food by inhibiting generation of ROS or by directly scavenging free radicals certain compounds may act *in vivo* as antioxidants by raising the levels of endogenous antioxidant defenses by up-regulating expression of the genes encoding superoxide dismutase (SOD), catalase or glutathione peroxide [3, 10].

Naturally occurring antioxidants in leafy vegetables and seeds such as vitamin C (ascorbic acid), vitamin E and phenolic compounds possess the ability to reduce the oxidative damage associated with many diseases including cancer, cardiovascular diseases, cataract, atherosclerosis, diabetes, arthritis, immune deficiency and ageing. In addition to this, free radical scavengers such as vitamin E, Beta-carotene and ascorbic acid act as secondary defense against ROS[4].

As numerous crude plants' extracts and pure natural compounds were previously reported to have antioxidant properties, many researchers have focused on natural antioxidants[4]. A plant containing flavonoids and phenolics has been reported to possess strong antioxidant properties. In the present study, *T. terrestris* was screened for *in vitro* antioxidant properties using standard procedures[4, 10].

MATERIALS AND METHODS

Plant material:

T. terrestris was collected from the local area of Sangli district of Maharashtra (India) in the month of September, 2012 and authenticated by Dr.G.G.Potdar, Assistant professor, Department of Botany, Yashwantrao Chavan College of Science, Vidyanagar, Karad, District - Satara, 415124 Maharashtra (India).

Preparation of extract:

The leaves of plant were washed, shade dried and powdered. The weighed powder (100g) was defatted with petroleum ether (60-80°C) and then extracted with ethanol using soxhlet apparatus (35 cycles each). The extract was concentrated for further studies at reduced pressure and temperature in a rotary evaporator. The defatted alcoholic extract was obtained from the starting material. Similar procedure was followed to prepare aqueous extract. Both the extracts were tested for presence of secondary metabolites by different phytochemical tests[4, 11].

Drugs and chemicals:

Ascorbic acid, DPPH (1,1-diphenyl -2-picryl-hydrazyl), Gallic acid, Folin–Ciocalteu reagent and Ethanol were obtained from Lobelia pvt. Ltd.

Preliminary Phytochemical Screening:

The leaf extract of *T.terrestris* was subjected to standard qualitative chemical tests for the detection of phytoconstituents, such as carbohydrates, glycosides, alkaloids, amino acids, phenolics, flavonoids, triterpenoids, steroids, etc[11].

Total Phenolic Contents:

Total phenolic content was determined according to a protocol similar to that of Kahkonen MP and et.al.[12]. *T.terrestris* alcoholic extract (10mg) was mixed with 1 ml of 95% ethanol, 5 ml of distilled water and 0.5 ml of 50% Folin–Ciocalteu reagent. The mixture was allowed to react for 5 min after which 1 ml of 5% Na_2CO_3 was added. Thereafter, it was thoroughly mixed and placed in dark for 1 hour and absorbance was measured at 725 nm using UV/Visible spectrophotometer. The amount of total phenolic contents was calculated as a Gallic acid equivalent from standard curve of Gallic acid solutions (covering the concentration range between 10 and 500µg/ml)[4, 12].

DPPH radical scavenging assay:

The DPPH radical scavenging activity assay used by Chan et al., 2007[13] and was adopted with slight modification. DPPH solution was prepared by dissolving 6mg of DPPH in 100ml of methanol. To 1ml of various concentrations of the extracts (10,20, 40,80,100 ug/ml), 2ml of DPPH solution (0.1mM) was added. An equal amount of methanol and DPPH served as control. The mixture was shaken vigorously and was left to stand in dark for 30 min. The absorbance of the resulting solution was measured spectrophotometrically at 517nm. Experiments were performed in triplicate and percentage scavenging activity of each extract on DPPH radical was calculated using the following formula:

$$\text{Scavenging activity (\%)} = \frac{1-\text{absorbance of the sample}}{\text{absorbance of control}} \text{x100}$$

DPPH radical scavenging activities of the extracts were expressed as IC_{50} values[13]. IC_{50}, the effective concentration of the extract required for 50% scavenging of DPPH radical was calculated from the graph of scavenging activity plotted against sample concentration using Microsoft Excel software.

Hydrogen peroxide scavenging activity:

Hydrogen peroxide scavenging activity of the extract was estimated by replacement titration. An aliquot of 1.0 ml of 0.1 mM H_2O_2 and 1.0 ml of various concentrations of extracts were mixed, followed by 2

drops of 3% ammonium molybdate, 10 ml of 2 M H_2SO_4 and 7.0 ml of 1.8M KI. The mixed solution was titrated with 5.09 mM NaS_2O_3 until yellow color disappeared. Percentage of scavenging of hydrogen peroxide was calculated as:

$$\% \text{ Inhibition} = (V_0 - V_1) / V_0 \text{ X } 100$$

Where V_0 was volume of NaS_2O_3 solution used to titrate the control sample in the presence of hydrogen peroxide (without extract), V_1 was the volume of NaS_2O_3 solution used in the presence of the extract[4].

STATISTICAL ANALYSIS

Tests were carried out in triplicate for all experiments. The amount of extract needed to inhibit free radicals concentration by 50% i.e. IC50 was graphically determined by a linear regression method using MS - Windows based Microsoft Office Excel 2007[4].

RESULT AND DISCUSSION

Synthetic antioxidants, such as butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA), have found limited use in foods as they are suspected to be carcinogenic. Therefore, the importance of search for natural antioxidants has greatly enhanced in the recent years[14].

Phytochemical screening:

Preliminary phytochemical screening of the *T.terrestris* leaf extract revealed the presence of different phytoconstituents which are represented in Table 1. Phytochemical screening of extract showed presence of saponin, flavonoids, lignin, alkaloids, steroids, carbohydrates, tannins, glycosides. Phytochemical analysis of alcoholic extract of *T. terrestris* revealed polyphenols. Total phenolic content of alcoholic extract of *T. terrestris* is a very high (0.325mg/100mg) as compared to aqueous extract (0.0251mg/100mg) of *T.terestris*. Polyphenols have been proved to possess antioxidant activities and have been used for the prevention and cure of various diseases which are mainly associated with free radicals[15,16].

Inhibition of DPPH radical:

DPPH is a free radical stable at ambient temperature and is often used to evaluate the antioxidant activity of several natural compounds. It accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Reduction capability of DPPH radicals is determined by the decrease in its absorbance at 517nm, which is induced by an antioxidant. Antioxidant on interaction with DPPH, either transfers electron or hydrogen atom to DPPH or neutralizes its free radical character. As this electron becomes paired in the presence of a free radical scavenger, absorption vanishes and resulting decolourisation is stoichiometric with respect to the number of electrons taken up[17]. As shown in figure1. *T. terrestris* exhibited concentration dependent scavenging activity by inhibiting DPPH radicals generated. The IC50 value of aqueous and alcoholic extract of *T. terrestris* were 43.96 ug/ml and 41.66 ug/ ml respectively.

Hydrogen peroxide scavenging activity:

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell hydrogen peroxide probably reacts with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate[4]. As shown in figure2, *T. terrestris* extract demonstrated hydrogen peroxide scavenging activity in a concentration dependent manner with the IC_{50} of the peroxide assay for aqueous extract and alcoholic extract 41.75ug/ml and 35.14ug/ml respectively.

CONCLUSION

Phytochemical screening of extract showed presence of saponin, flavonoids and lignin, alkaloids, steroids, carbohydrates, tannins, glycosides. The results also showed that it is a rich source of polyphenols which led to the conclusion that high soluble phenolics in the *T. terrestris* extract could be taken into account for the strong free radical scavenging activity. It is suggested that further work should be performed on the isolation and identification of the individual antioxidant components in *T. terrestris***.**

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Table 1
Preliminary phytochemical screening of *T.terestris* leaf extract.

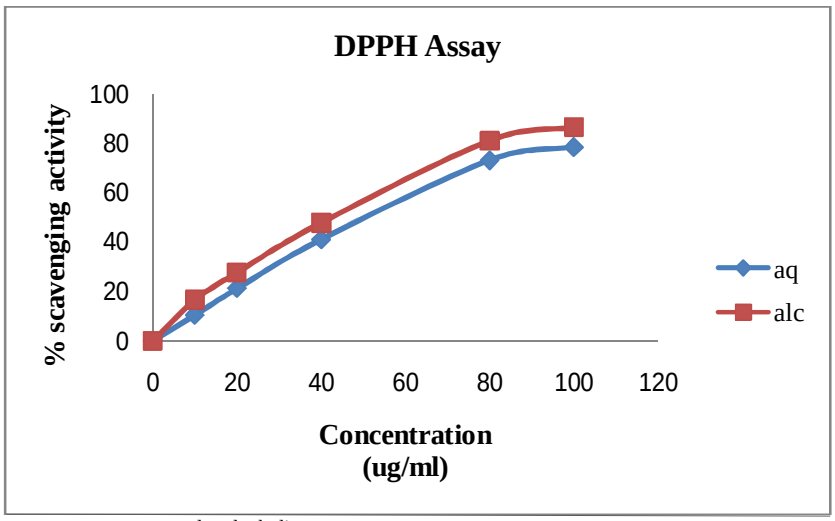
Phytoconstituents	Test performed	Result
Alkaloids	Dragendroff's test Mayer's, Wagner's and Hager's test	+ve +ve
Flavonoids	Alkaline reagent and Shinoda test	+ve
Steroids	Libermann Burchard and Salkowski test	+ve
Triterpenoids	Libermann Burchard and Salkowski test	-ve
Carbohydrates	Molisch's and Fehling's test Barfoed's and Benedict's test	+ve +ve
Amino acids	Millon's and Ninhydrin test	-ve
Tannins	FeCl2 test	+ve
Saponin	Foam test	+ve
Glycosides	Killer-Kiliani test	+ve

(+)ve: Indicates the presence of chemical constituents. (-)ve: Indicates the absence of chemical constituents.

Table2
Radical scavenging activity of aqueous and alcoholic extracts of *T. terrestris* at different concentrations

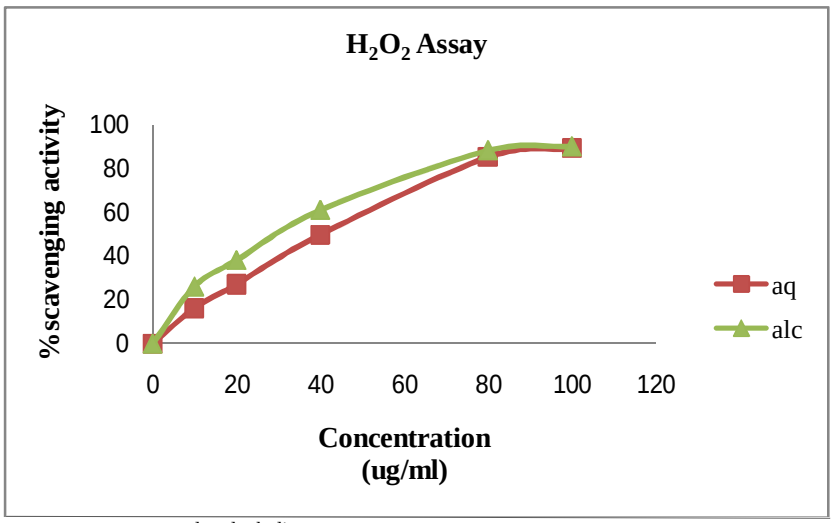
Concentration (ug/ml)	DPPH radical scavenging %		H_2O_2 scavenging	
	Aqueous extract	Alcoholic extract	Aqueous extract	Alcoholic extract
10	10.55	16.66	16.24	26.12
20	21.32	27.77	27.15	38.11
40	41.11	47.88	49.77	61
80	73.23	81.13	85.24	88.21
100	78.66	86.65	89.24	89.99
IC50 for extract	43.96(0.98)*	41.66(0.97)*	41.75(0.97)*	35.14(0.91)*
IC50 for ascorbic acid	19.66(0.99)*	19.66(0.99)*	18.03(0.97)*	18.03(0.97)*

Here r*- regression coefficient



aq: aqueous extract, alc: alcoholic extract

Figure 1
DPPH radical scavenging activity of aqueous and alcoholic extract of *T.terrestris* at different concentrations



aq: aqueous extract, alc: alcoholic extract

Figure 2
Hydrogen Peroxide Scavenging Activity by using extracts of *T. terrestris*

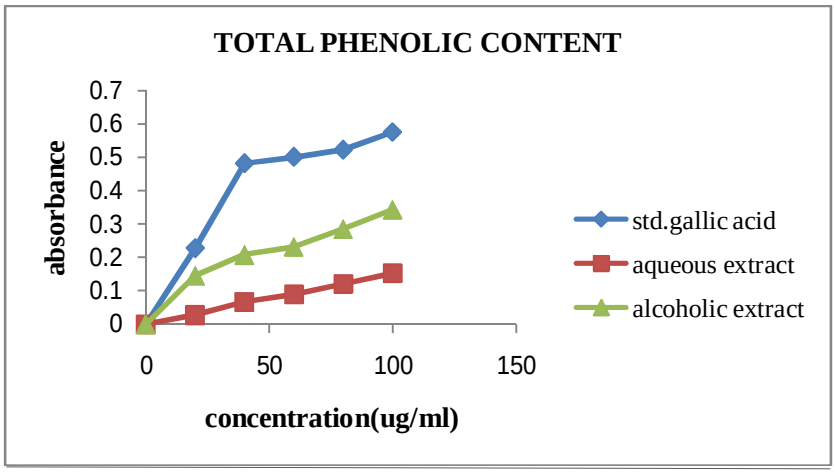


Figure 3
Total phenolic contents of extracts of *T. terrestris* .

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