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ANTIOXIDANT ACTIVITY BY DPPH RADICAL SCAVENGING METHOD OF METHANOLIC EXTRACT OF *n*-BUTANOL FRACTION OF *TRIBULUS TERRESTRIS* LINN. (FAMILY ZYGOPHYLLACEAE)

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Keywords:

Tribulus terrestris L., Antioxidant activity, DPPH, Toluene and *n*-butanol

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ABSTRACT: In this study antioxidant activity was performed by DPPH (1,1 diphenyl-2-picrylhydrazyl) radical scavenging method for *Tribulus terrestris* L. whole plant methanolic extraction fractionation with toluene and *n*-butanol in succession. The obtained fractions were concentrated under reduced pressure to yield corresponding antioxidant activity. The IC_{50} concentration for the standard, ascorbic acid and BF-TTME was found to be 0.085 and 4.5 µg/ml respectively.

INTRODUCTION: Antioxidants play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables [1]. Plant-sourced antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, *etc.* have been recognized as having the potential to reduce disease risk [2].

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Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties [3]. A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity [4]. The DPPH assay method is based on the reduction of DPPH, a stable free radical [5].

The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple color). When Antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (*e.g.*, a free radical

scavenging antioxidant) and is reduced to the DPPHH and as a consequence the absorbance's decreased from the DPPH [6]. Radical to the DPPH-H form results in decolorization (yellow color) concerning the number of electrons captured [7].

More the decolorization more is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug [8]. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (Diphenylpicrylhydrazine; non radical) with the loss of this violet color (although there would be expected to be a residual pale yellow color from the picryl group still present) [9]. This plant has been reported to possess antioxidant properties. So, this study has been undertaken to *Tribulus terrestris* Linn. (Zygophyllaceae) (TT) popularly known as puncture vine is a perennial creeping herb with a worldwide distribution.

Since, ancient times it is regarded as an aphrodisiac in addition to its beneficial claims on various ailments such as urinary infections, inflammations, edema and ascites [10]. *Tribulus terrestris* growing in Bulgaria is a source for the industrial production of the original preparation "TribestanTM" produced by Sopharma AD, Bulgaria. TribestanTM consists of the *n*-BuOH extract of the aerial parts of the same plant and is successfully applied for treatment of sexual deficiency [11].

The active components of TribestanTM are steroid saponins of furostanol. The dominating furostanol bisglycosides have been identified as protodioscin and protogracillin [12]. An intensive screening on qualitative and quantitative composition of raw materials from TT and a variety of preparations from different origin demonstrated that Bulgarian preparation TribestanTM contains the highest amount of protodioscin and protogracillin [13]. The aphrodisiac property of TT extract was explored in castrated rats [14]. Administration of TT to humans and animals improves libido and spermatogenesis [15]. Protodioscin is also found to increase the levels of testosterone, luteinizing hormone [16], dehydroepiandrosterone [17], dihydrotestosterone and dehydroepiandrosterone sulfate [18]. Clinical studies showed TT improved reproductive function, including increased concentration of hormones such as estradiol, with testosterone being very slightly influenced, thereby improving reproductive function, libido and ovulation [19].

Free radicals are a group of highly reactive chemical molecules with one or more unpaired electrons that can oxidatively modify biomolecules they encounter. Reacting almost immediately with any substance in their surrounding area, they begin a chain reaction leading to cellular damage [20]. Oxidative damage, caused by reactive oxygen species (ROS), has been frequently associated with the pathogenesis of various conditions such as arthritis, cancer, inflammation, heart diseases [21] and contributes to defective spermatogenesis leading to male factor infertility [22]. Several clinical trials have examined the potential of an antioxidant such as carnitines, vitamin E, vitamin C, selenium, carotenoids, *etc.* supplementation to treat oxidative stress-induced male factor infertility [23]. The present study aimed to investigate free radical scavenging and antioxidant activity of TT fractional preparations using DPPH scavenging method.

MATERIALS AND METHODS:

Collection and Preparation of Extracts: The plant *Tribulus terrestris* Linn. was collected in December 2010, from rice fields of Warangal, Telangana India, after the authentication of the plant by Prof. V. S. Raju, Department of Botany, Kakatiya University, Warangal. The air-dried whole plant material was coarsely powdered and macerated with methanol in a round bottom flask for 7 days with intermittent stirring and filtered after seven days and concentrated under reduced pressure to yield a green semisolid mass. It was given a code TTME. The obtained TTME was suspended in water and fractionated with toluene and n-butyl alcohol in succession. The obtained fractions were concentrated under reduced pressure to yield corresponding extracts. They were given the codes, as TF-TTME (Toluene fraction), BF-TTME (n-butyl alcohol fraction) and AF-TTME (Aqueous fraction- the residue left in the water after the fractionation process).

Chemical: 1,1-Diphenyl-2-picrylhydrazyl (DPPH), (UV-Spectrophotometer; Elico-SL 159, Germany). Ascorbic acid (Trolox TM) was from Sigma-Aldrich USA. All the other chemicals used including the solvents were of analytical grade. All

solvents were of HPLC grade and were purchased from Merck (Darmstadt, Germany) or Sigma-Aldrich (St. Louis, MO).

Determination of Antioxidant Activity of BF-TTME by DPPH Free Radical Scavenging Assay: Free radical scavenging activity of test extract was measured by *in-vitro* method using DPPH [13]. 0.1 mM solution of DPPH in methanol was prepared and 1ml of this solution was added to 2.5ml of test extract suspension in water at different concentrations (10, 20, 40, 60, 80, 100 µg/ml). The reaction mixture was then allowed to stand at room temperature in a dark chamber for 30 min. After 30 min, absorbance was measured at 517 nm on a spectrophotometer (UV-Spectro-photometer; Elico-SL 159, Germany). The percentage inhibition of different concentrations was calculated by comparing the absorbance values of control and samples. The concentration of the fraction required to decrease the initial concentration by 50% (IC_{50}) was calculated.

DPPH scavenging effect (%) or Percent inhibition = $A_0 - A_1 / A_0 \times 100$.

Where, A_0 was the Absorbance of control reaction and A_1 was the Absorbance in the presence of a test or standard sample.

Statistical Analysis: The data obtained were analyzed by one-way of variance (ANOVA) followed by Student-Newman-Keul multiple comparison tests for the significant interrelation between the various groups using Graph pad prism-3 in stat computer software. $P<0.05$ was considered to be significant from the toxic.

RESULTS:

DPPH Free Radical Scavenging Assay of BF-TTME: The whole plant methanolic extract fractions of this plant showed better antioxidant potential when compare 1, 1 Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of the BF-TTME and ascorbic acid are summarized in **Table 1**.

TABLE 1: DPPH FREE RADICAL SCAVENGING ASSAY OF BF- *TRIBULUS TERRESTRIS*

	Concentration (µG/ML)	%Inhibition	IC_{50}values (µg/ml)
Ascorbic Acid	0.010	20.5	
	0.020	28.4	
	0.040	46.1	
	0.060	57.1	
	0.080	70.5	
	0.1	92.1	0.085
BF-TT	1	31.4	
	2	40.4	
	4	61.0	
	6	73.1	
	8	87.4	
	10	96.5	4.5

Both the fraction and ascorbic acid exhibited a concentration-dependent DPPH radical scavenging activity. The IC_{50} concentration for the standard, ascorbic acid and for BF-TTME was found to be 0.085 and 4.5 µg/ml respectively.

DISCUSSION AND CONCLUSION: This study determined that methanolic extract of a butanol fraction of *Tribulus terrestris* Linn. showed better antioxidant potential by DPPH radical scavenging method when compared to standard ascorbic acid 19 and the IC_{50} concentration for the standard, ascorbic acid and for BF-TTME were found to be 0.085 and 4.5 µg/ml respectively. So, we can say this butanol fraction has antioxidant activity.

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CONFLICT OF INTEREST: Nil

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