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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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ABSTRACT: In this study Anti oxidant activity was performed by DPPH(1,1 diphenyl-2-picryl hydrazyl) 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 IC50 concentration for the standard, ascorbic acid and for BF-TTME were found to be 0.085 and 4.5 µg/ml respectively.

INTRODUCTION: Antioxidants play an important role as 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.

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 517nm (purple colour). 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 DPPH and as a consequence the absorbance is decreased from the DPPH⁶. Radical to the DPPH-H form, results in decolorization (yellow colour) with respect to 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 colour (although there would be expected to be a residual pale yellow colour 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, oedema and ascites¹⁰. *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 deficiency¹¹.

The active components of Tribestan TM are steroid saponins of furostanol. The dominating furostanol bisglycosides have been identified as protodioscin and protogracillin¹². An intensive screening on qualitative and quantitative composition of raw materials from TT and variety of preparations from different origin demonstrated that Bulgarian preparation TribestanTM contains the highest amount of protodioscin and protogracillin¹³. The aphrodisiac property of TT extract was explored in castrated rats¹⁴. Administration of TT to humans and animals improves libido and spermatogenesis¹⁵. Protodioscin is also found to increase the levels of testosterone, leutinizing hormone¹⁶, dehydroepiandrosterone¹⁷, dihydrotestosterone and dehydroepiandrosterone sulphate¹⁸. 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 ovulation¹⁹. 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 damage²⁰. Oxidative damage, caused by reactive oxygen species (ROS), has been frequently associated with the pathogenesis of various conditions such as arthritis, cancer, inflammation, heart diseases²¹ and contributes to defective spermatogenesis leading to male factor infertility²². Several clinical trials have examined the potential of antioxidant such as carnitines, Vitamin E, Vitamin C, selenium, carotenoids etc. supplementation to treat oxidative stress-induced male factor infertility²³.

The aim of the present study was 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 the month of 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 fractionation process).

Chemical: 1,1-Diphenyl-2-picrylhydrazyl (DPPH), (UV-Spectrophotometer; Elico-SL 159, Germany). Ascorbic acid (Trolox TM) were 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 DPPH¹³. 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 517nm on a spectrophotometer (UV-Spectrophotometer; 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% (IC₅₀) was calculated.

DPPH scavenging effect (%) or

$$\text{Percent inhibition} = A_0 - A_1 / A_0 \times 100.$$

Where A₀ was the Absorbance of control reaction and A₁ was the Absorbance in presence of test or standard sample.

Statistical analysis: The data obtained were analyzed by one-way of variance (ANOVA) followed by Student-Newman-Keul multiple comparison test 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-picryl hydrazyl (DPPH) free radical scavenging activity of the BF-TTME and ascorbic acid are summarized in **Table 1**. Both the fraction and ascorbic acid exhibited a concentration dependant DPPH radical scavenging activity. The IC₅₀ concentration for the standard, ascorbic acid and for BF-TTME were found to be 0.085 and 4.5 µg/ml respectively.

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

DISCUSSION: This study determined that methanolic extract of butanol fraction of *Tribulus terrestris* Linn. showed better antioxidant potential by DPPH radical scavenging method when compare to standard ascorbic acid 19 and the IC₅₀ 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 is having antioxidant activity.

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