Int J Pharm Bio Sci 2013 Oct; 4(4): (B) 784 - 793

Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

COMPARATIVE ANTIOXIDANT ACTIVITIES OF CRUDE ETHANOLIC AND SAPONIN RICH BUTANOL EXTRACTS OF *TRIBULUS TERRESTRIS* FRUITS

S.HEMALATHA AND RAJESWARI HARI*

Department of Biotechnology, Dr. M.G.R. Educational and Research Institute, University, Maduravoyal, Chennai,Tamil Nadu, India.

ABSTRACT

A comparative free radical scavenging activity of ethanolic crude extract (EETT) and its saponin rich butanol fraction (SFTT) of *Tribulus terrestris* was investigated. The total antioxidant activity and the reducing capacity were also compared. The ethanolic crude extract and the saponin rich butanol fractions were prepared and its ability to scavenge free radicals such as nitric oxide, hydroxyl radical and hydrogen peroxide were investigated employing various established *in-vitro* systems. The total antioxidant activity was estimated using phosphomolybdenum method. The total reducing capacity was also estimated using potassium ferric cyanide method. The results revealed that the saponin rich butanol extract has notable quenching of nitric oxide, hydroxyl radical and hydrogen peroxide radicals when compared to the ethanolic crude extract of *Tribulus terrestris.* The SFTT at 1mg/ml showed maximum scavenging of nitric oxide (90.30%) hydroxyl (90.02%) and hydrogen peroxide (89.00%) against the scavenging of EETT which showed nitric (76.19%) hydroxyl (74.31%) and hydrogen peroxide (51.26%) radicals respectively at the same concentration. A linear correlation between these extracts and reducing power and total antioxidant activity were observed with SFTT showing pronounced activity than the EETT. The results of this study strongly indicate that the SFTT has more potent antioxidant activity than the crude EETT.

KEYWORDS: **Antioxidant, Free radicals, Saponins, *Tribulus terrestris***

RAJESWARI HARI
Department of Biotechnology, Dr. M.G.R. Educational and Research Institute, University, Maduravoyal, Chennai,Tamil Nadu, India.

INTRODUCTION

Oxidative stress is due to imbalance between the generation of the oxygen derived radicals such as superoxide, hydrogen peroxide, hydroxyl and nitric oxide and the organism's endogenous antioxidant potential to counteract them[1]. There is an increasing evidence which has shown the involvement of these Reactive Oxygen Species (ROS) in a variety of diseases, that can cause damage to the cellular biomolecules such as nucleic acids, protein, lipids and carbohydrates and leads to accelerated cellular oxidation causingcardiovascular disease, tumour growth, wrinkled skin, cancer, Alzheimer's disease, and even adecline in energy and endurance[2,3]. Natural antioxidants present in food of plant origin on the other hand interfere with the chain reaction by removing free radical intermediates and inhibit other oxidation reactionspreserving good health[4,5].Some synthetic antioxidants, such as butylated hydroxylanisole (BHA) and butylated hydroxytoluene (BTH), exhibit potent free radical scavenging effects,but they exert side effects .Hence there has been an increased interest in the food industry and in preventive medicine in the development of "Natural antioxidants" from plant materials[6,7,8]. Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity and protect against harmful diseases[9,10].*Tribulus terrestris* L.which belongs to the family Zygophyllaceae is commonly known as caltrops or devil's thorn, Gokshura in Sanskrit and Nerunji in Tamil, is found throughout the world[11]. In several countries *Tribulus terrestris* is used as folk medicine such as tonic, aphrodisiac, astringent, analgesic, stomachic, anti-hypertensive, diuretic and urinary anti-septic[12]. Historically, *Tribulus terrestris* was used by the cultures of India and Greece as a rejuvenation tonic [13]. The plant has been extensively investigated by a number of works, for the presence of steroidal glycosides, steroidal, saponins, flavonoids and alkaloids[14].Saponin-containing herbs possess a broad range of bioactivities and have been commonly used in folk medicine for their health-promoting properties .The aim of the present study is to investigate free radical scavenging and antioxidant activity of the saponin mixture from *Tribulus terrestris*.

MATERIALS AND METHODS

(i)Chemicals and Reagent

Rutin , Vitamin E, ascorbic acid, mannitol, deoxyribose and EDTA were obtained from Sigma Chemical Company, St. Louis, USA. Sodium nitroprusside, thiobarbituric acid and H_2O_2were obtained from Sd Fine Chemicals Ltd., India. All the other chemicals and reagents used in this study were of analytical grade.

(ii)Collection of Plant Material

The ripe fruits of*Tribulus terrestris* were collected from the Sairam siddha Medical College and research center India and were authenticated by Dr. Sankaranarayanan, Assistant Director, Dept of Research and Development, of the same college. The voucher specimen is also available in herbarium file of the same center.

(iii)Preparation of Ethanolic Extract of Tribulus terrestris (EETT)

The fruits of *Tribulus terrestris* (500 g) were shade-dried and pulverized to a coarse powder. The powder was passed through 40-mesh sieve and exhaustively extracted with 90% ethanol by cold maceration for 48 hrs at room temperature.The extract was evaporated under pressure till all the solvent had been removed and further removal of water was carried out by freeze drying to give an extract sample with yield of 10.56% (w/w).The extract was stored in refrigerator and a weighed amount of the extract was dissolved in 10 % (v/v) aqueous poly ethylene glycol and used for the present investigation.

(iv) Preparation ofSaponin rich Fraction of Tribulus terrestris (SFTT)

The saponins isolation was carried out according to the method of Kostova etal., 2002[15].The fruits of *Tribulus terrestris* (500 g) were shade-dried and pulverized to a coarse powder and was extracted with 70% ethanol (3 × 24 h) at room temperature.The combined ethanol solutions were concentrated to a small volume and extracted in succession with chloroform (3 × 24 h) and n-BuOH (3 × 24 h). The n-BuOH layer was concentrated to dryness to give crude saponin extract. The presences of saponins were confirmed by frothing test (0.5 ml filtrate + 5 ml distilled water). The saponins content was calculated as, a percentage of total saponins=weight of residue ×100⁄weight of sample taken. The yield of the saponins prepared by the above procedure was found to be 0.34 %(w/w). All the extracts obtained were stored at 4^0C in air tight containers until assay.

(v)Determination of Free Radicals Scavenging Activity

(a)Nitric oxide scavenging activity

Nitric oxide scavenging activity was estimated by the method of Marcocciet al., (1994)[16].Various concentration of the extracts were mixed with sodiumnitroprusside in PBS and a final volume of 3 ml was incubated at 25˚C for 150min. After incubation, 0.5 ml of sample was removed and diluted with 0.5 ml of Griess reagent and the absorbance was read at 546nm.Rutin was used as positive control under the same assay conditions.

Nitric oxide scavenging activity (%) = [(A_o-A1 ⁄ A_o) × 100]

Where A_o is the absorbance of the control and A_1 is the absorbance of plant extract or the standard sample.

(b)Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity was determined according to the method of Halliwell et al. (1987)[17].The reaction mixture containing 0.1 ml deoxyribose, 0.1 ml $FeCl_3$, 0.1 ml ascorbic acid, 0.1 ml EDTA, and 0.1 ml H_2O_2 were mixed with various concentrations of the extracts in 1 ml of final volume made with KH_2PO_4-KOH buffer pH 7.4 and was incubated in a water bath at 37˚C for 1 hr. The extent of deoxyribose degradation was measured by adding 1 ml of TBA and 1 ml trichloroacetic acid and the mixture and heated at 100˚C for 20 min. After cooling to room temperature the absorbance was measured at 532 nm. Mannitol,was used as positive control. The hydroxyl radical scavenging activity was calculated using the following formula:

Hydroxyl radical scavenging activity (%) [(A_o-A1⁄A_o)×100]

Where A_o is the absorbance of the control and A_1 is the absorbance of plant extract or the standard sample.

(c)Hydrogen Peroxide Scavenging Activity

The ability of samples to quench H_2O_2 was determined by Ruch et al. (1989)[18].The samples were dissolved in 3.4 ml of phosphate buffered saline (PBS) and mixed with 0.6 ml of 2 mM solution of H_2O_2. Absorbance of H_2O_2 at 230 nm was determined 10 min later in a spectrophotometer.Vitamin E was used as the standard.Hydrogen peroxide radical scavenging activity(%) = [(A_o-A1⁄A_o)×100], where A_o is the absorbance of the control, and A1 is the absorbance of BHE or the standard sample.

(d)Reducing Power Assay

The reducing power of the prepared extract was determined according to method of Oyaizu (1986)[19]. The various concentrations of the extract and the standard compound (BHT) in 1 ml of distilled water were mixed with 2.5 ml of phosphate buffer and 2.5 ml of a potassium ferricyanide solution. The mixture was incubated in a water bath at 50˚C for 20 min. Then 2.5 ml of a 10% TCA solution was added and the mixture was then centrifuged at 3000g for 10 min. 2.5 ml aliquot of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of a ferric chloride solution, the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture correlates with greater reducing power.

(e)Test for Ferric Ion Reducing Capacity

The ferric ion reducing capacity was determined according to the method of Wang et al. (2003)[20] with minor modifications. Here different concentration of the extracts was mixed with 200µl of 20mM phosphate buffer pH 6.5 and 100µl of ferric chloride (2mM). The mixture was incubated for 30 min. At the end of the incubation 1ml of potassium thiocyanate

(4mM) was added and absorbance of ferric-thiocyanate complex (reddish brown complex) was measured at 460 nm using spectrophotometer. The results were compared with standard EDTA which were treated similarly.

(f)Total Antioxidant Capacity

The total antioxidant capacity was measured by spectrophotometric method of prietoet al[21].The different concentrations of EETT and SFTT ranging from 100-1000µgms were mixed with 0.6M H_2So_4 and 28mM of sodium phosphate and , 4mM ammonium molybdate mixture. The tubes were incubated for 90 mins at 95˚C. The mixture was cooled to room temperature and the absorbance was read at 695 nm against blank.Ascorbic acid at the same concentrations were used as a standard The total antioxidant activity were expressed as equivalents of ascorbic acid in µg per mg of extract.

(vi)Statistical Analysis

The experimental results were mean ± SEM of three parallel measurements. IC_{50} values were obtained through linear regression analysis. Mean differences were analysed statistically by running one-way analysis of variance test (ANOVA).*P*< 0.05 was considered statistically significant.

RESULTS

Nitric Oxide Scavenging Activity

Table -I shows the nitric oxide scavenging activity of the EETT, SFTT and the positive control Rutin. All these three extracts scavenged the nitric oxide in a concentration dependent manner.However pronounced scavenging activity is observed in the saponin fraction of the *T. terrestris* (p<0.05) when comparable to its ethanolic crude extract.

Table I

Nitric oxide scavenging activity of different plant extracts

Concentration in µgms / ml	Inhibitory activity of EETT (%)	Inhibitory activity of SFTT (%)	Inhibitory activity of Rutin (%)
100	10.54±1.25a*	12.89 ± 3.38b*	16.25±5.94
200	24.87±3.49a*	26.60 ± 4.73b*	30.51±8.92
400	36.95±2.36a*	37.16 ± 8.33b*	42.05±5.61
500	41.46±5.68a**	49.83 ± 1.37b*	54.73±2.34
600	58.15±3.27a*	64.36 ± 0.33b^{ns}	65.53±1.68
800	67.94±4.18a**	76.83 ± 0.56b*	79.98±6.39
1000	76.19±7.59a**	90.30 ± 0.45b^{ns}	91.46±2.64

Nitric oxide radical scavenging activity of EETT, SFTT and Rutin.
Each value represents the mean ± SEM (n = 3).
Comparison between
A - EETT vs. Rutin
b - SFTT vs. Rutin
****p<0.05, * *p<0.01, NS–Not Significant***

At concentrations of 800and 1000 µgmsthe nitric oxide inhibitory effect of the saponin fraction was 76.83% and 90.30% respectively against the positive control rutin which showed 79.98% and 91.46% inhibition at the same concentration. However the EC_{50} value for saponin fraction was found to be less that is 250µg/ml when comparable to the positive control rutin which showed much higher value of EC_{50} value that is 550µg/ml.

Hydroxyl Radical Scavenging

There was a marked reduction in thedeoxyribose cleavage induced by hydroxyl radicals by the plant extract in the present study. All the three extracts scavenged the hydroxyl radicals in a concentration dependent manner. The hydroxyl radical scavenging activity of crude extract, saponin extract and standard mannitol were shown in the Table II.

Table II
Hydroxyl radical scavenging activity of different plant extracts

Concentration in µgms / ml	Inhibitory activity of EETT (%)	Inhibitory activity of SFTT (%)	Inhibitory activity of Mannitol (%)
100	12.61±5.26a*	17.20 ± 4.77b^{ns}	18.68±6.81
200	24.83±2.58a*	27.51 ± 1.23b^{ns}	28.20±2.30
400	34.44±2.49a*	36.15 ± 2.41b^{ns}	37.60±2.41
500	41.22±8.136a*	44.10 ± 3.00b^{ns}	45.24±4.15
600	50.82±2.49a**	61.83 ± 4.13b^{ns}	62.40±3.82
800	64.50±8.16a**	79.12 ± 1.84b^{ns}	82.80±1.72
1000	74.31±1.27a**	90.02 ± 2.48b^{ns}	91.4±2.84

Hydroxyl radical scavenging activity of EETT, SFTT and Mannitol.
Each value represents the mean ± SEM (n = 3).
Comparison between
A - EETTvsMannitol
B - SFTTvsMannitol
****p<0.05, * *p<0.01, NS–Not Significant***

The hydroxyl radical scavenging activity was more for the saponins fraction when compared (p<0.01) to ethanolic crude extract. In the present investigation it has been observed that saponins and Mannitol showed similar inhibitory activity starting from the lower concentration of 100µg/ml. Butfor the saponin fraction EC_{50} value in scavenging the hydroxyl radical was found to be 530µg/ml against the positive control Mannitol which showed a minimum EC_{50} value of 210µg/ml in scavenging these radicals.

Hydrogen Peroxide Radical Scavenging

Table-IIIdepictsthehydrogen peroxide radical scavenging activity of the two extracts along with the positive control Vitamin E.Inthepresentinvestigationalltheplantextractss cavengedthehydrogen peroxide radical considerably. In correlation with nitric and hydroxyl radical scavenging activity the SFTT effectively scavenged the hydrogen peroxide radicals than its crude extract EETT.

Table III
Hydrogen peroxide radical scavenging activity of different plant extracts

Concentration in µgms / ml	Inhibitory activity of EETT (%)	Inhibitory activity of SFTT (%)	Inhibitory activity of Vitamin E (%)
100	10.68±5.99a**	13.33±4.33b**	27.89±0.89
200	15.35±2.35a**	23.33±5.04b*	34.96±0.56
400	24.32±4.94a**	35.66±3.75b*	41.95±0.35
500	34.38±8.51a**	49.66±4.17b^{ns}	49.92±0.82
600	41.64±2.34a**	57.00±3.05b*	62.12±0.14
800	43.58±9.15a**	77.00±5.50b**	85.31±0.39
1000	51.26±9.61a**	89.00±2.72b*	98.71±0.78

Hydrogen peroxide radical scavenging activity of EETT, SFTT and Vitamin E.
Each value represents the mean ± SEM (n = 3).
Comparison between
a - EETTvsVitamin E
b - SFTTvs Vitamin E
****p<0.05, * *p<0.01, NS–Not Significant***

Attheconcentrationof1mg/ml the percentage inhibition of hydrogen peroxide radicals by SFTT was 89.00 and that of EETT was 51.26 against the positive control vitamin E which showed about 98.71% inhibition of hydrogen peroxide radicals at the same concentration.

Reducing Power Assay

Table IV shows the reducing power of the *Tribulus terrestris* ethanolic crude extract (EETT), its saponin fraction(SFTT) and BHT, as a function of their concentrations. In this assay it has been observed that the reducing power of the crude EETT was almost similar to the positive control BHT and the saponin fraction showed much higher reducing capacity

than the positive control BHT. With this assay the saponin fraction contains 123mg/g of BHT equivalents whereas the amount of BHT equivalents present in crude EETT was found to be 53mg/g.

Table VI
Reducing power of the different plant extracts

Concentration in µgms / ml	Absorbance of EETT in (O.D)	Absorbance of SFTT in (O.D)	Absorbance of BHT in (O.D)
100	0.26±0.06a*	0.38 ± 0.01b*	0.30±0.03
200	0.34±0.09a*	0.40 ± 0.08b^{ns}	0.38±0.03
400	0.41±0.07a*	0.46 ± 0.02b^{ns}	0.49±0.02
500	0.52±0.03a*	0.57 ± 0.01b^{ns}	0.58±0.57
600	0.59±0.05a^{ns}	0.67 ± 0.02b*	0.62±0.20
800	0.68±0.04a*	0.72 ± 0.08b^{ns}	0.75±0.05
1000	0.71±0.09a**	0.80 ± 0.03b*	0.90±0.34

Reducing capacity of EETT,SFTTand standard BHT. Each value represents the mean ± SEM (n = 3).
Comparison between
a - BHT vsEETT
b - BHT vs.SFTT
****p<0.05, * *p<0.01***

Test for Ferric Ion Reducing Capacity

In the present investigation significant results were obtained from EETT,SFTT and EDTA on Fe^{3+} metal chelating activity. Table V shows the chelating ability of the different extracts on metal transition ions (Fe^{3+}) that increases in a dose dependent manner when compared with the control.

Table V
Ferric Ion chelating activity of the different plant extracts

Concentration in µgms / ml	Chelation of Fe^{3+} EETT (%)	Chelation of Fe^{3+} SFTT (%)	Chelation of Fe^{3+} EDTA (%)
100	13.54 ± 0.02a**	22.32±0.08b*	29.32±0.08
200	15.13 ± 0.06a**	28.59±0.25b*	34.72±0.08
400	24.82 ± 0.13a**	35.13±0.08b**	48.94±0.01
500	27.94 ± 0.08a**	41.21±0.08b**	57.25±0.53
600	29.25 ± 0.07a**	51.95±0.04b*	60.38±0.07
800	31.75 ± 0.11a**	55.09±0.05b**	69.64±0.06
1000	34.49 ±0.06a**	59.17±0.67b***	85.42±0.06

Ferric Ion chelating activity of the EETT,SFTT and standard BHT. Each value represents the mean ± SEM (n = 3).
Comparison between
a - BHT vs EETT
b - BHT vs. SFTT
****p<0.05, * *p<0.01***

It is observed that the saponin fraction of *T.terrestris* exhibited higher chelating activity than the crude ethanolic extract of *T.terrestris.*At the concentration of 1mg/ml SFTT exhibited 59.17% chelation whereas EETT showed only 34.49 % chelation. However this chelation was found to be much lower when comparable to the positive control EDTA which showed 85.42% of chelation at the same concentration.Based on these results, it might be concluded that these extracts act as an electron donor capable of neutralizing free radicals.

Total Antioxidant Capacity

The antioxidant capacity of the fractions was measured spectrophotometrically through phosphomolybdenum method, which was based on the reduction of Mo (VI) to Mo (V) with a maximum absorption at 695 nm.Lower absorbance indicates a higher level of antioxidant activity.

Table VI
Total antioxidant activity of the different plant extracts

Concentration in µgms / ml	Absorbance of EETT in (O.D)	Absorbance of SFTT in (O.D)	Absorbance of ascorbic acid (O.D)
100	0.75±0.05a**	0.90±0.05b^{ns}	0.92±0.07
200	0.63±0.06a**	0.81±0.07b^{ns}	0.84±0.02
400	0.57±0.08a**	0.74±0.15b^{ns}	0.79±0.04
500	0.51±0.01a**	0.64±0.25b*	0.71±0.03
600	0.48±0.19a**	0.61±0.04b^{ns}	0.64±0.01
800	0.28±0.17a*	0.39±0.08b^{ns}	0.42±0.02
1000	0.12±0.02a*	0.18±0.01b^{ns}	0.22±0.04

Total antioxidant activity of theEETT,SFTTand standard BHT. Each value represents the mean ± SEM (n = 3). Comparison between
a - BHT vsEETT
b - BHT vs. SFTT
****p<0.05, * *p<0.01***

The antioxidant capacity was found to be very high for the saponinfraction of *T. terrestris* (SFTT) extract when compared to crude extract of *T. terrestris* which increases with increase in concentration.The antioxidant activity of the saponin fraction was similar to that of the positive control ascorbic acid.From the above experiments it was estimated that the SFTT was found to contain 67µg of ascorbic acid/mg of extract whereas the crude extract of T.*terrestris*contain 32.5µg of ascorbic acid/mg of extract.

DISCUSSION

The alterations in the oxidant and the antioxidant profile are known to be involved in the pathophysiology, thus affecting the cell and its components, causing damage to them and releasing their products as markers [22] Epidemiological studies show that the consumption of vegetables and fruits can protect humans against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species [23]There are numerous methods available to study the antioxidant activity of the plant extracts. In the present investigation the free radicals such as nitric oxide , hydrogen peroxide and hydroxyl radical scavenging activities of the plant extract was studied. In addition the reducing capacity, metal chelating power and its total antioxidant activity were also investigated. Nitric oxide or reactive nitrogen species, formed during its reaction with oxygen or with superoxide, such as NO_2, N_2O_4, N_3O_4, nitrate and nitrite are very reactive. These compounds alter the structure and function of many cellular components. Nitric oxide, because of its unpaired electron, is classified as a free radical and displays important reactivity's with certain types of proteins and other free radicals[24].In the present study the saponin fraction of *T. Trerrestris* was very effective in scavenging the nitric oxide radicals when comparable to the ethanolic extract of *T. trerrestris*. Saponins are steroid or triterpenoid glycosides, common in a large number of plants and plant products that are important in human and animal nutrition. Saponin-containing herbs possess a broad range of bioactivities and have been commonly used in folk medicine for their health-promoting properties. *T.terrestris* is one such saponin-containing herb used from high antiquity to energize, vitalize, and improve sexual function and physical performance in men[25]. We examined the inhibitory action of the ethanol as well as the saponin extract fraction on deoxyribose degradation which gives an indication of hydroxyl radical scavenging activity [26].Our results clearly demonstrated the capacity of the two extracts to quench hydroxyl radicals with saponin fraction exhibiting higher scavenging activity. Moreover, hydroxyl radicals are capable of the quick initiation of lipid peroxidation process as by abstracting hydrogen atoms from unsaturated fatty acids. Saponin extract was more effective in scavenging the hydroxyl radicals when compared with crude extract which seems to be directly related to the prevention of propagation of lipid peroxidation. This may be attributed due to the presence of pharmacological active metabolites like furostanol and spi- rostanolsaponins[27].The results of this study clearly indicate that the plant extracts not only scavenges off free

radical but also inhibits the generation of free radicals. Hydrogen peroxide is formed by two-electron reduction of O_3 which is not a free radical, but an oxidizing agent. In the presence of O_3 and transition metal ions, the H_2O_2 can generate OH radical via Fenton reaction. Mallakckakronet al., (2004)[28]reported, that in addition, H_2O_2 can easily cross the cell membrane and exerts an injurious effect on tissues through a number of different mechanisms such as, perturbing intracellular Ca^{2+} homeostasis, increasing intracellular ATP, inducing DNA damage, and cell apoptosis (So the removal of H_2O_2 is important for the antioxidant defence mechanism. In Comparing ethanol extract and the saponin fraction effectively scavenged the hydrogen peroxide radicals. Chen and Jiang et al have reported the presence of eight saponins

namelyhecogenin-3-*O*-b –Dglucopyranosyl (1→4)-b -D-galactopyranoside , tigogenin- 3-*O*-b -D-glucopyranosyl (1→4)-b -D-galactopyranoside, hecogenin-3-*O*-b -D-glucopyranosyl(1→2)-b -D-glucopyranosyl(1→4)-b -D-galactopyranoside , hecogenin-3-*O*-b -D-xylopyranosyl(1→3)-b -D-glucopyranosyl(1→4)-b -D-galactopyranoside , tigogenin-3-*O*-b -D-xylopyranosyl(1→2)-[b -D-xylopyranosyl-(1→3)]-b -D-glucopyranosyl(1→4)-[a -L-rhamnopyranosyl(1→2)]-b -D-galactopyranoside , 3-*O*-{b -D-xylopyranosyl(1→2)-[b -D-xylopyranosyl(1→3)]-b -D-glucopyranosyl(1→4)-[a -L-rhamnopyranosyl(1→2)]-b -D-galactopyranosyl}26-*O*-b -D-glucopyranosyl-22-methoxy-(3b ,5a ,25*R*)-furostan-3,26-diol, hecogenin-3-*O*b -D-glucopyranosyl(1→2)-[b -D-xylopyranosyl(1→3)]-b -D-glucopyranosyl(1→4)-b -D-galactopyranoside, tigogenin-3-*O*-b -D-glucopyranosyl(1→2)-[b -D-xylopyranosy l(1→3)] -b -D-glucopyranosyl(1→4)-b -D-galactopyranoside[29] respectivelyare responsible for the free radical scavenging activity observed in our present investigation.

Hsu etal.,[30]in his findings stated that the reducing capacity of compound may serve as a significant indicator of its potential antioxidant activity.The reducing power of *Tribulus terrestris* fractions increased and correlated well with the increasing concentration. However, as anticipated, the reduction power of saponin fraction was relatively higher than the ethanol fraction of *Tribulusterrestris*. It is observed in the present investigation that the reducing capacity of the saponin fraction was more pronounced than the BHT which is used as the positive control. Saponins are steroid or triterpenoid glycosides, common in a large number of plants and plant products that are important because of their pharmacological actions.Saponins such as glucopyranosyl, galactopyrans, ruscogin, hecogenin, gitogenin, titogenin, protodioscin, diosgening and yamogenin exhibit diverse biological functions[31]..These bioactive agents contains many ring structures with reducing capability responsible for the reducing power observed in the present investigation. The total antioxidant activities of the plant extract were measured using phosphomolybdenum method, which determines the amount of peroxide produced at the initial stage of lipid peroxidation. Lower absorbance indicates a higher level of antioxidant activity. Among the constituents that contributed to biological activity of the seeds steroid saponin glycosides(astrogalosides) play a major role[32].According to Jiang *et al.* (2007)[33]generally O-glycosides contain one or more of the hydroxyl groups which are bound to sugars. Recently, more than fifty steroidal saponins have been isolated from this plant[34,35, 36]. The above-mentioned effects might be expected for the saponin rich fraction in reducing the lipid peroxidation.

CONCLUSION

The obtained results suggest that the saponin rich fraction Tribulus terrestris butanolfraction can form a good source of effective inhibitors of free radicals.further investigations regarding their phytoactive compounds is needed.

REFERENCES

1. Abdollahi M, Ranjbar A, Shadnia S, Nikfar S andRezaiee A, Pesticides and oxidative stress: A review. Med. Sci. Monit, 10: 141-47,(2004).
2. Fresquet F, Pourageaud F, Leblais V, Brandes RP, Savineau JP, Marthan R and Muller B, Role of reactive oxygen species and gp91phox in endothelial dysfunction of pulmonary arteries induced by chronic hypoxia. Brit. J. Pharmacol, 148(5): 714-723,(2006).
3. Lata H andAhuja G K, Role of free radicals in health and disease.Ind. J. Physio and Allied Sci, 57: 124-128, (2003).
4. Seis H, Strategies of antioxidant defence. Eur. J. Biochem,215 (2):213-9, (1993).
5. Seal T, Antioxidant activity of some wild edible plants of Meghalaya state of India: A comparison using two solvent extraction systems. Int. J.Nutrand Meta, 4: 51-56, (2012).
6. Imaida K, Fukushima S, Shirui T, Ohtani M, Nakanishi K and ItoN,In vitro antioxidantactivites of Aloe vera leaf skin.Carcinogenesis,*4:* 895, (1983).
7. Jadhav SJ, Nimbalkar SS, Kulkarni AD and Madhavi DL, Lipid oxidation in biological andfood systems. In: Madhavi D L, Deshpande S S andSalunkhe D K, editors. FoodAntioxidants: Technological, Toxicological, andHealth Perspectives. New York, NY: Marcel Dekker. 5-63, (1996).
8. Hirose M,Takesada Y, Tanaka H,Tamano S, Kato T and Shirai T,Carcinogenicity of antioxidants BHA, caffeic acid, sesamol, 4-methoxyphenoland catechol at low doses, either alone or in combination and modulation oftheir effects in a rat medium-term multi-organ carcinogenesis model.Carcinogenesis, 19 (1): 207-12, (1998).
9. Zheng W and Wang SY, Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem, 49(11):5165-5170, (2001).
10. Cai YZ, Sun M and Corke H, Antioxidant activity of betalains from plants of theAmaranthaceae. J Agric Food Chem, 51(8):2288-2294, (2003).
11. Tholkappian B,Charles martin A and Narayanan N, Quality control aspects of the whole plant caltrop. International Journal of Pharma and Bio science,vol 2, 593, (2011).
12. Ashwanikumar,Comparative and Quantitative determination of quercetin and other flavonoids in north indian populations of *Tribulus terrestris*by HPLC.International Journal of Pharma and Bio science, 3(4): 69-79, (2012).
13. Adaikan PG, Gauthaman K, Prasad RN, NgSC, Proerectile pharmacological effectsof Tribulus terrestris extract on the rabbit corpuscavernosum. Ann. Acad. Med. Singapore, 29(1):22-26, (2000).
14. SengprachaW, M.Sc. Thesis, University of Wollongong, Wollongong, 78, (2001).
15. IvankaKostovaa,DragomirDincheva, Gudrun HoppRentschb, Vladimir Dimitrovb, andAntoanetaIvanovaa,Two New SulfatedFurostanol Saponins fromTribulus terrestris. Z Natur.forsch C, 57(1-2):33-8, (2002).
16. Marcocci L, Maguire JJ, Droy-Lefaix MT and Packer L,The nitricoxide-scavenging properties of Ginkgo biloba extract (EGb 761). Biochem.Biophys. Res.Commun, 201: 748-755, (1994).
17. Halliwell B, Gutteridge JMC andAruoma OI, The deoxyribose method: A simple “test tube”assay for determination of rate constants for reaction of hydroxyl radicals. Anal Biochem,165: 215-219,(1987).
18. Ruch KJ, Cheng SJ and Klauning JE,Prevention of cytotoxicity and inhibitionof intercellular communication by antioxidant catechin isolated from Chinese green tea. Carcinogenesis, 10:1003-1008, (1989).
19. Oyaizu M,Studies on products of browning reaction prepared from glucoseamine. Jap J Nutr, 44:307-315,(1986).
20. Wang L, Yen JH, Ling HL and Wu MJ,Antioxidant effect of methanolextracts from lotus plumule and blossom

*(NelumbonuciferaGertn).*J FoodDrug Anal, 11:60-66,(2003).
21. Prieto P, Pineda M and Aguilar MM, Spectrophotometric quantification of antioxidant capacity through the formation of a phoshomolybdenumcomplex;specific application to the determination of vitamin E. Anal.Biochem,269:337-341,(1999).
22. Valko M, Rhodes CJ, Moncol J, Izakovic M and Mazur M, Free radicals, metals,and antioxidants in oxidative stress induced cancer. Chem. Biol. Interact,18:31-35,(2006).
23. Ames BM, Shigena MK and Hagen TM, Oxidants, antioxidants and thedegenerative diseases of aging. Proc. Natl. AcadSci. USA, 90: 7915 – 7922,(1993).
24. Babu BH, Shylesh BS and Padikkala J, Antioxidant and hepatoprotective effectof Alanthusicicifocus.Fitoterapia, 72: 272-277,(2001).
25. Neychev VK, Nikolova E, Zhelev N and Mitev VI, Saponins from Tribulus terrestris L. Are Less Toxic for Normal Human Fibroblasts than for Many Cancer Lines: Influence on Apoptosis and Proliferation .Experimental Biology and Medicine, 232:126-133,(2007).
26. Lopes GK, Schulman HM and Hermes-Lima M, Polyphenol tannic acid inhibits formation from Fenton reaction by complexing ferrous ion. Biochim.Biphys.Acta, 1472: 142-152,(1999).
27. Kostova I and Dinchev D, Saponins in Tribulus terrestris-chemistry andbioactivity. Phytochem. Rev, 4 (2-3): 111–137,(2005).
28. Mallakckakorn P, Tollda M, Mcrakami Y, Matsumoto K and Watanabe H, Antioxidant and free radical-scavenging activity of Choto-san and its related constituents. Bio. Pharm.Bulle,27: 38,(2004).
29. Jun-Dong ZHANG,*a*Yong-Bing CAO,*a*ZhengXU,*a*Hui-HuaSUN,*c*Mao-Mao An,*a*LanYAN,*a*Hai-Sheng CHEN,*,*b* Ping-HuiGAO,*a*Yan WANG,*a*Xin-Ming JIA,*a*and Yuan-Ying JIANG*,*a In Vitro* and *in Vivo* Antifungal Activities of the Eight Steroid Saponins from *Tribulus terrestris* L. with Potent Activityagainst Fluconazole-Resistant ,Fungal Biol. Pharm. Bull,28(12): 2211—2215, (2005).
30. Hsu B, Coupar IM and Ng K, Antioxidant activity of hot water extract from the fruit of the Doum palm, Hyphaenethebaica. Food.Chem, 98: 317-328,(2006).
31. Xuy X, Chen H S, Liamg H Q, Gu Z B, Lui W Y, Leung W N and Lit J,Three new saponins from Tribulus terrestris. PlantaMedica, 66(6): 545-550,(2001).
32. Ganzera M ,Bedir E and Khan IA, Determination of steroidal saponins in Tribulus terrestris by reverse phase high performance liquid chromatography and evaporative light scattering detection . Journal of pharmaceutical sciences , 90 : 1752-1758,(2001).
33. Jiang P, Burczynski C, Campbell G, Pierce JA, Austria CJ and Briggs,Rutin and flavonoid contents in three buckwheat species Fagopyrumesculentum, F. tataricum, and F. homotropicum and their protective effects against lipid peroxidation .Food.Res. Int ,40:356-364,(2007).
34. Wang, Y, Kazuhiro O, Ryoji K and Kazho Y, Steroidal saponins from fruits of Tribulus terrestris.Phytochemistry,42: 1417–1422,(1996).
35. Huang JW, Jiang S H, Tan CH and Zhu DY, Structural elucidation of three new Steroidsapogenins.Chin. J. Org. Chem,22, 917–921,(2002).
36. Huang JW, Jiang SH, Tan CH and Zhu DY,Sapogenins from Tribulus terrestris. Nat. Producr. Res. Dev,15: 101–103,(2003).