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Preliminary Phytochemical Screening and *in vitro* Antioxidant Potential of Hydro-Ethanolic extract of *Euphorbia neriifolia* Linn

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Abstract: Oxidative stress induced ROS and free radicals are believed to be major cause of degenerative diseases. Naturally occurring antioxidant supplements from plants are vital to counter the oxidative damage in cells. We assessed the antioxidant potential and phytochemical constituents of crude hydro-alcoholic extract of *Euphorbia neriifolia* (EN) using tests involving inhibition of DPPH, H_2O_2, superoxide anions, reducing power, FRAP and metal chelating activities. The phenolic, flavonoid and tannin contents of the extract were also determined using standard phytochemical reaction methods. EN extract showed the presence of alkaloids, tannins, saponins, flavonoids and cardiac glycosides. A positive correlation between the antioxidant activities and physiochemical assays was observed and the highest scavenging activity of extract was noticed at concentration of 1mg/ml. The percentage inhibition of lipid peroxide at the initial stage of oxidation showed antioxidant activity of 76.15 % compared to those of ascorbic acid (75.6%), BHA (60.8%) and BHT (75.6%). The percentage inhibition of metal chelating capacity of extract and standard was found to be 73.24% and 85.37% respectively. Our findings reveals that the hydro-alcoholic extract of EN leaves possess antioxidant properties and could serve as free radical inhibitors or scavenger or, acting possibly as natural antioxidants and this justified its uses as anti-inflammatory, anti-analgesic, anti-anemic, anticancer in folkloric medicines.
Keywords: Antioxidant, *Euphorbia neriifolia*, DPPH, FRAP, BHT.

INTRODUCTION

Oxidative stress induced ROS and free radicals are believed to be major cause of physiological disorders like Alzheimers, Parkinson's, Arthritis, Atherosclerosis, coronary heart diseases, Emphysema, gastric ulcer, diabetes mellitus, cirrhosis, aging and cancer[1, 2]. ROS, are highly reactive molecules which include free radicals such as superoxide ions (O_2^-), hydroxyl radicals (OH^-), nitric oxide radical (NO), singlet molecular oxygen peroxynitrite radicals and hydrogen peroxide (H_2O_2). Superoxide anion radical ($O_2^{\cdot-}$) is one of the strongest reactive oxygen species among free radicals that are generated first after oxygen is taken into living cells[3]. Although synthetic antioxidant like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propylgallate (PG) and tertiary butyl-hydroquinone (TBHQ) are known to ameliorate oxidative damages but they are suspected to have some toxic effects[4] prompting research for the determination, development and utilization of more effective antioxidants of natural origin that have significant scavenging properties and are less toxic and inherently safer than synthetic antioxidants[5].

Presence of a multitudes of vitamins, polyphenols, flavonoids, tannins and phenolic acids in natural extracts of vegetables, fruits, herbs, spices and medicinal plants and inverse relationship between these natural antioxidants and the risk of oxidative diseases has caused spurt in extensive research and have been described to possess biological activities such as antioxidant, anti-inflammatory, oestrogenic, cytotoxic, antitumoural[6].

Euphorbia neriifolia Linn (Family: Euphorbiaceae) commonly known as "Sehund or thohar" in Hindi, is

found throughout the Deccan Peninsula of India and grows luxuriously around the dry, hilly, rocky areas of North, Central and South India. *E. neriifolia* is easily available in large quantity and can be used as a cheap source of active therapeutics, as propagation of this plant is easy and cheap which can be grown in large number with very less expenses. Leaves are thick succulent, 6-12 inch long, ovular in shape. Ayurveda describes the plant as bitter, pungent, laxative, carminative, improves appetite useful in abdominal troubles, bronchitis, tumors, loss of consciousness, delirium, leucoderma, piles, inflammation, enlargement of spleen, anaemia, ulcers and fever. As far as our literature survey could ascertain, no information was available on the *in vitro* antioxidant activities of the *E. neriifolia*.

Objective of this study were to investigate the phytoconstituents, antioxidant and free radical scavenging potential of hydro-ethanolic extract of *Euphorbia neriifolia* leaves. The antioxidant activities of *Euphorbia neriifolia* (EN) were measured in a concentration range of 0.1-1mg/ml (100 – 1000 µg/ml), using different antioxidant assays. Furthermore, the total phenolics, flavonoids and tannins contents were also measured and their correlation with the antioxidant activities was ascertained.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

Folin-Ciocalteus's reagent, HCl, Dragendorff's reagent, methanol, gallic acid, commercial saponins, H_2SO_4, Na_2CO_3, vanillin, aluminium chloride, potassium acetate, potassium persulphate, sodium nitroprusside, hydrogen peroxide, sulfanilic acid, glacial acetic acid, naphthylethylenediamine dichloride, NADH were all purchased from Merck, USA. DPPH (1,1-diphenyl-1,2-picryl hydrazyl), TPTZ (2,4,6,-tripyridyl-s-triazine), Ferrozine, Deoxyribose were purchased from Sigma Chemical Co. Ltd USA. Trichloroacetic acid (TCA), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), L-Ascorbic acid, ammonium molybdate, nitroblue tetrazolium (NBT), PMS (phenazine methosulfate), reduced NADH (nicotinamide adenine dinucleotide), quercetin were purchased from HI Media, Mumbai. All other unlabelled chemicals and reagents were of analytical grade and of highest purity (≥ 99.0%).

PLANT MATERIAL

Euphorbia neriifolia leaves were collected from Pharmacological garden of Banasthali University, Banasthali, India, in the month of September 2009. The plant was identified with the help of available literature and authenticated by Botanist of Krishi Vigyan Kendra, Banasthali Vidyapith, Banasthali, Tonk district.

PREPARATION OF HYDRO-ETHANOLIC CRUDE EXTRACT

Freshly collected *Euphorbia neriifolia* leaves were dried in shade and coarse powder was extracted by macerating 500 g in 1.5 L of ethanol (70% v/v) for one week with occasional stirring. The macerated mixture was filtered through muslin cloth and evaporated at 40°C up to one third of initial volume, remaining solvent was completely evaporated at 40°C, using a hot air oven (Mvtex, India) and kept in dissector for two days. The yield of the extract was 20% w/w of the powdered plant material. Collected the dried extract and stored at 5°C in air-tight container. The residue was designated as hydro-ethanolic extract and used for further studies.

QUALITATIVE PHYTOCHEMICAL SCREENING

The freshly prepared extract of EN leaves was qualitatively tested for the presence of phytochemical which includes alkaloids, flavonoids, tannins, phenolics, terpenoids, saponins and steroids in accordance with the methods described by Parekh, & Chanda[7]: Test for alkaloids performed with Dragendorffs reagent, flavonoids with the use of ammonia and concentrated H_2SO_4, tannins with ferric chloride and potassium dichromate solutions, phenolics with $FeCl_3$, Saponins (frothing test), Steroids (Liebermann-Burchard test), terpenoids with Fehling's solution, Cardiac glycosides (Keller-Kinliani test).

Table 1: Qualitative Phytochemical Screening of *Euphorbia neriifolia* (EN) Extract.

Phytochemicals	*Euphorbia neriifolia*
Alkaloid	++
Terpenoids	+
Tannin	++
Saponin	+++
Steroid	–
Flavonoid	+
Phenolic	–
Phlobatannins	+
Cardiac glycosides	++

(+++) appreciable amount; (++) moderate amount; (+) trace amount; (-) completely absent

QUANTITATIVE PHYSICO-CHEMICAL ASSAYS

DETERMINATION OF TOTAL PHENOLIC CONTENT

The total phenolic content of plant extract was determined using Folin-Ciocalteu reagent[8]. To 1 ml of Folin-Ciocalteu's reagent, previously diluted (1:20) was added to 1 ml of samples (250µg/ml) and mixed thoroughly. To the mixture, 4 ml of sodium carbonate (75 g/L) and 10 ml of distilled water were added and mixed well. The mixture was allowed to stand for 2 h at room temperature. Contents were then centrifuged at 2000 g for 5 min and the absorbance of the supernatant was taken at 760 nm. All determinations were carried out in triplicates. A standard curve was obtained using various concentrations of gallic acid. Samples of extract were evaluated at a final concentration of 1mg/ml. Total content of phenolic compounds in plant extract in gallic acid equivalents (GAE) was calculated by the following formula:

$C = c. V/m'$

Where: C- Total content of phenolic compounds, mg/g plant extract (GAE),
c-The concentration of gallic acid established from the calibration curve (mg/ml), V- The volume of extract (ml),
m- The weight of pure plant extract (g).

DETERMINATION OF TOTAL FLAVONOIDS

Total flavonoids were measured by $AlCl_3$ colorimetric assay[9]. To 0.5 ml of sample, 0.5 ml of 2% $AlCl_3$ ethanol solution was added. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 1mg/ml. All determinations were carried out in triplicates. The amount of flavonoids in plant extract in rutin equivalent (RE) was calculated by the following formula:

$X= (A. m_0)/ (A_0. m)$

Where,
X- The total flavonoid content, mg/mg plant extract in RE,
A- The absorption of plant extract solution,
A_0- The absorption of standard rutin solution,
m- The weight of plant extract (mg)
m_0- The weight of rutin in the solution, (mg)

DETERMINATION OF TOTAL TANNIN

Tannin content was determined by Vanillin hydrochloride method of Sadasivam, & Maickam[10]. Vanillin hydrochloride reagent was prepared by mixing equal volumes of 8% HCl in methanol and 4% vanillin in methanol. A volume of 1.0 ml of 0.1 mg/ml of extract solution was mixed with 5 ml vanillin hydrochloride reagent; the mixture was allowed to stand for 20 min. The absorbance was measured at 500 nm. Extract samples were evaluated at a final concentration of 1 mg/ml. Total tannin content were expressed as rutin equivalents (mg/g) using the following equation based on calibration curve: y = ax + b, where x was the absorbance and y was the rutin equivalent (mg/g).

EVALUATION OF ANTIOXIDANT ASSAY

DPPH FREE RADICAL SCAVENGING ACTIVITY

The free-radical scavenging activity of extract was measured by decrease in the absorbance of methanol solution of DPPH[11]. A stock solution of DPPH (33 mg in 1 L) was prepared in methanol, which gave initial absorbance of 0.493, and 5ml of this stock solution was added to 1 ml of EN leaves extract solution at different concentrations (100–1000 µg/ml). After 30 min, absorbance was measured at 517 nm and compared with standards (100-1000 µg/ml). Scavenging activity was expressed as the percentage inhibition calculated using the following formula:

$$\% \text{ Anti-radical activity} = \frac{\text{Control Abs - Sample Abs}}{\text{Control Abs}} \times 100$$

SCAVENGING OF HYDROGEN PEROXIDE

The ability of extract to scavenge hydrogen peroxide was determined according to the method of Ilhami et al., [12]. A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). Different concentrations (100–1000 µg/ml) of EN leaves extract were added to a hydrogen peroxide solution (0.6 ml, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min. against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage inhibition of hydrogen peroxide of EN and standard compounds was calculated using the following formula:

$\%$ inhibition $[H_2O_2] = [(A_0\text{-}A1)/A_0] \times 100$

Where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the sample of EN and standards.

SUPEROXIDE RADICAL ($O_2^{\circ -}$) SCAVENGING ACTIVITY

The effect of hydro-alcoholic extract of EN leaves on superoxide radical production (SRP) was evaluated following the nitro blue tetrazolium (NBT) reduction method of Shivkumar et al., [13]. The reaction mixture consisting of 1ml of nitro blue tetrazolium (NBT) solution (144µM in 100mM phosphate buffer, pH 7.4),

1 ml NADH solution (677µM in100mM phosphate buffer, pH 7.4), and 0.5 ml of sample extract was mixed and the reaction was started by adding 100 µl of phenazine methosulfate (PMS) solution (60 µM PMS in 100mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 min and the absorbance was measured at 560 nm against blank sample and compared with standards. Decreased absorbance of reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

% inhibition = $[(A_0\text{-}A1)/A_0] \times 100$

REDUCING POWER

The reducing power of EN leaves was determined as per the reported method of Oyaizu[14]. Different concentrations of extract (100–1000 µg/ml) in 1ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferrocyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm and compared with standards. Increased absorbance of the reaction mixture indicated increased reducing power.

TOTAL ANTIOXIDANT ACTIVITY (FRAP ASSAY)

A modified method of Benzie, & Strain [15] was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml $FeCl_3 \cdot 6H_2O$. The temperature of the solution was raised to 37°C before using. Plant extract (150 µL) was allowed to react with 2850 µl of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $FeSO_4$. Results were expressed in µM Fe (II)/g dry mass and compared with that of BHT and quercetin.

METAL CHELATING ACTIVITY ASSAY

The chelating activity of the extracts for ferrous ions Fe^{2+} was measured according to the method of Dinis et al., [16]. To 0.5 mL of extract, 1.6 mL of deionized water and 0.05 mL of $FeCl_2$ (2 mM) was added. After 30 s, 0.1 mL ferrozine (5 mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min at room temperature, the absorbance of the Fe^{2+}–Ferrozine complex was measured at 562 nm. The chelating activity of the extract for Fe2+ was calculated as Chelating rate (%) = $(A_0 - A_1) / A_0 \times 100$ Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

STATISTICAL ANALYSIS

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. The data were subjected to one-way analysis of variance (ANOVA) and differences between samples were determined by Bonferroni's multiple comparison tests using the SPSS 16.0 (Statistical program for Social Sciences) program. Results with $p<0.05$ were regarded as statistically significant and considered $p<0.001$ as very significant. Pearson correlation analysis was performed between antioxidant activity and total phenolic content.

Table 2: Extraction Yield and Total Amount of Plant Phenolic Compounds, Flavonoids, and Tannin of EN.

Plant	Yield %	Total phenols mg/g plant extract (GAE)	Total flavonoids mg/g plant extract (RE)	Total Tannin mg/g plant extract (RE)
EN	20	0.60±0.09*	1.50±0.21*	0.98±0.06*

*P- Value <0.001 Vs standard group, Bonferrni test.

Table 3: Radical Scavenging Activities of Hydro-Alcoholic Extract of EN.

Sample tested	DPPH radical scavenging (%)	Reducing power activity (absorbance)	Hydrogen peroxide radical scavenging (%)	Superoxide radical scavenging activity (%)
EN	76.2 ± 0.07*	0.095	69.0 ± 0.01*	50.1 ± 0.06*
Ascorbic acid	75.6 ± 0.19*	0.032	12.7± 0.21*	60.6± 0.05*
BHA	60.8 ± 0.19*	—	—	36.4± 0.11*
BHT	75.6 ± 0.02*	0.516	44.7 ± 0.06*	42.6± 0.12*
Rutin	—	—	—	73.1 ± 0.2*

Data are presented as the mean±SD of each triplicate test.

*P- Value <0.001 Vs standard group, Bonferrni test.

Table 4: Representing the Metal Chelating inhibition (%) and Total Antioxidant (FRAP) Assays of EN Extract.

Sample tested	Metal chelating % Inhibition	FRAP (µmol Fe(II)/g
EN	73.24±0.019*	149.2±0.05*
BHT	–	333.1±0.01*
EDTA	85.37±0.025*	_
Quercetin	–	43.8±0.06*

Data are presented as the mean±SD of each triplicate test.

*P- Value <0.001 Vs standard group, Bonferrni test.

Fig1. DPPH Scavenging Activity of EN Extract at different Concentrations

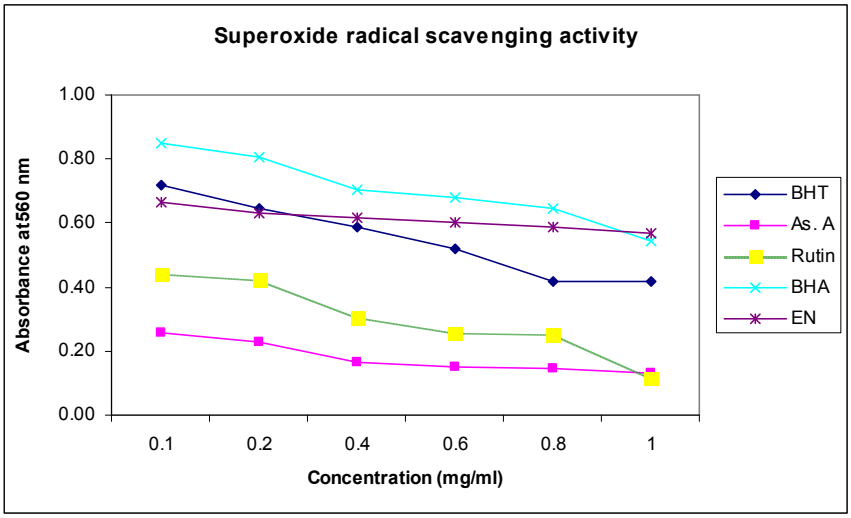


Fig.2. Hydrogen Peroxide Scavenging Activity of EN Ethanol Extract at different Concentrations

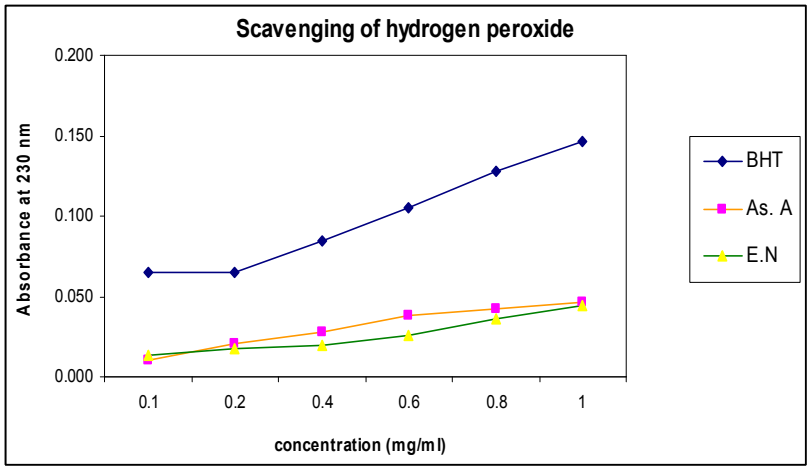
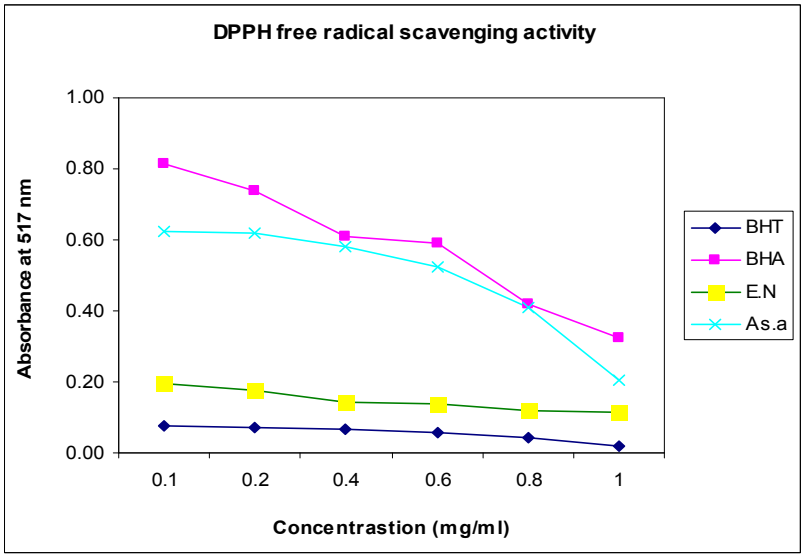


Fig.3. Superoxide Radical Scavenging Activity of EN Extract at different Concentrations



RESULTS AND DISCUSSION

QUALITATIVE PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening of the hydro-ethanolic extract of *Euphorbia neriifolia* leaves revealed the presence of various bioactive components of which alkaloid, saponin, tannin and cardiac glycosides were the most prominent and the result of phytochemical test has been summarized in Table 1. All these phytochemicals possess good antioxidant activities and has been reported to exhibit multiple biological effects including anti-inflammatory, antitumor activities. *Euphorbia neriifolia* is tested negative for steroid and phlobatannins. These results are in support with the observations made by Bigoniya, & Rana [17]. Sapogenin, a novel saponin was isolated from the leaves of *Euphorbia neriifolia*, it has already been established to possess good haemolytic and *in vitro* antioxidant activity and it exhibits cytotoxic activity on murine F_1B16 melanoma cell lines was confirmed already. The presence of phenolics like flavonoids and tannins in the extract of EN act as primary antioxidants or free radical scavengers.

THE AMOUNT OF PHENOLIC COMPOUNDS, FLAVONOIDS AND TANNINS CONTENT

The yield of extract and the total antioxidant capacity of total phenolic, flavonoids, and tannins content (mg/g of dry material) are shown in Table 2. The amount of the extract obtained from the extraction was 20g (20 % w/w yield).

Plant phenolics are the widest spread secondary metabolite in plant kingdom. These compounds have received much attention as potential natural antioxidant in terms of their ability to act as both efficient radical scavengers and metal chelator. Therefore, it is worthwhile to determine the total

amount of phenolic content in the plant chosen for the study. The content of phenolic compounds (mg/g) in plant extract, determined from regression equation of calibration curve (y = 0.124x +0.214, R^2 = 0.26) and expressed in gallic acid equivalent (GAE).

Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolics[18]. The total flavonoid content (mg/g) in EN extract, determined from regression equation of calibration curve (y = 1.114x+ 0.847, R^2 = 0.967) and expressed in rutin equivalent (RE).

Our present investigation depicts high content of saponin and flavonoids in the plant extract as compared to other phenolic compounds.

It can be observed that the content of phenolics in the extract correlates with the Reducing power, (r= 0.963). Results obtained in the present study reveals that the level of all these compounds in extract was significantly higher ($p<0.001$) as compared to the reference standard used for this study.

Polyphenol are the major plant compounds and are commonly found in both edible and inedible plants and they have been reported to have multiple biological effects, including antioxidant activity. Their antioxidant activity is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[19]. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers [20].

DPPH RADICAL SCAVENGING ACTIVITY

Free radical assay is one of the most widely used method and has become routine in establishing the antioxidant activity of herbal extracts and phytochemicals. Hydrogen donating ability is an index of primary antioxidants. DPPH is known to abstract labile hydrogen and the ability to scavenge the DPPH radical is related to the inhibition of lipid peroxidation [21]. DPPH radical was used as a substrate to evaluate free radical scavenging activity of EN extract. DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for deep purple color. When DPPH accept an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. BHT, ascorbic acid and BHA were used as standards. At concentration of 1mg/ml the scavenging activity of EN extract reached to 76.15%. On the other hand the ascorbic acid, BHT and BHA at same concentration had 75.6%, 75.6% and 60.8% of scavenging activity. The data presented in Table 3 depicts that the extract possess the scavenging character in accordance with the standards and Figure 1 shows their activities at different concentration. The study reveals that the extract of *Euphorbia neriifolia* exhibits the proton- donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. These observations are in accordance with [20, 22].

SCAVENGING OF HYDROGEN PEROXIDE

Hydrogen peroxide scavenging activity of the EN extract on hydroxyl radical is given in Table 3 and compared with the standards, ascorbic acid and BHT and Figure 2 shows their activities at different concentration. H_2O_2 is highly important because of its ability to penetrate biological membranes. H_2O_2 itself is not very reactive, it is a weak oxidizing agent, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. It can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react 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 can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate.

Thus, removal of H_2O_2 is very important for protection of food systems. EN extract also demonstrated hydrogen peroxide decomposition activity in a concentration dependent manner. The decomposition of H_2O_2 by hydro-alcoholic extract of EN may at least partly result from its antioxidant and free radical scavenging activity. The correlation between total flavonoids content and hydrogen peroxide in this study was found positive (r = 0.932). The percentage of H_2O_2 scavenging activity of EN extract and the standard used i.e. ascorbic acid and BHT was found to be 69.01%, 12.7% and 44.7%, respectively.

SUPEROXIDE RADICAL SCAVENGING ASSAY

Superoxide anions are one of the most representative free radicals that can damage biomolecules directly or indirectly by forming H_2O_2, OH, peroxynitrite or singlet oxygen during aging and pathological events such as ischemic reperfusion injury. Superoxides are produced from molecular oxygen due to oxidative enzymes [23] of body as well as via non-enzymatic reaction such as autoxidation by catecholamines. The superoxide anion radical scavenging activity of EN extract assayed by the PMS-NADH system is shown in Table 3 and Figure 3 shows their activities at different concentration.

The percentage inhibition of superoxide generation at 1mg/ml concentration of extract was found to be

50.06%. On the other hand, BHT, BHA, rutin and ascorbic acid at 1mg/ml concentration had 42.6%, 36.36%, 73.08% and 60.55% inhibition of superoxide radical. Thus increase of % scavenging activity thus indicates the consumption of superoxide anion in the reaction mixture by plant extract.

The result clearly indicates that EN extract inhibits free radicals in dose dependent manner and suggests that the plant extract exhibited potent antioxidant effect *in vitro* and can serve as good candidate for further evaluation of its bio-efficacies, active constituents and bimolecular mechanisms *in vitro* as well as *in vivo*. Our findings are in conformation with [20].

REDUCING POWER

Reducing power is to measure the reductive ability of antioxidant and it is evaluated by the transformation of Fe^{3+} to Fe^{2+} by donating an electron, in the presence of the ethanol extract of *Euphorbia neriifolia*. Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, and prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [24, 25].

Like the antioxidant capacity, the reductive power of ethanol extract increase with rising concentrations (0.1 to 1 mg/ml) and the increasing absorbance at 700 nm indicates an increase in reductive ability. Our data (Table 3) on the reducing activity of the extract suggest that it is likely to contribute significantly towards the ($p<0.001$) observed antioxidant effect. Our observation indicates that there is a good correlation between the reducing power and the total phenolic content ($r= 0.963$). The result shows that extract consist of hydrophilic polyphenolic compounds that cause the greater reducing power. The good reducing power of sapogenin means that triterpenoidal compounds, especially euphol, are electron donors and therefore, can act as antioxidants [26].

FRAP ASSAY

The ability of plant extract to reduce ferric ions was determined in FRAP assay. The change in absorbance at 593 nm owing to the formation of blue colored Fe^{+2}-tripyridyltriiazine (TPTZ) compound from the colourless oxidized Fe^{+3} form by the action of electron donating antioxidants. The FRAP values of extract was found to be significantly higher as compared to the standards i.e. quercetin and BHT (1mg/ml) is given in Table 4. Since FRAP assay is easily reproducible and linearly related to molar concentration of the antioxidant present, thus it can be reported that extract of EN may act as free radical scavenger, capable of transforming reactive free radical species into stable non radical products.

The antioxidant potentials of the extract of the leaves of EN was estimated from their ability to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II) at 593 nm and its antioxidant activity increased proportionally with the polyphenol content.

METAL CHELATING ACTIVITY

Ferrous ion can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals [27]. Ferrozine can make complexes with ferrous ions. In the presence of chelating agents, complex (red colored) formation is interrupted and as a result, the red color of the complex is decreased. Thus, the chelating effect of the coexisting chelator can be determined by measuring the rate of color reduction. In this assay the extract and standard compound interfered with the formation of the ferrozine– Fe^{2+} complex suggesting that it has chelating activity and captures the ferrous ion before ferrozine. The absorbance of Fe^{2+}–ferrozine complex is linearly decreased with the increasing concentration (0.1 to 1 mg/ml). The % of metal chelating capacity of extract and standard was found to be 73.24% and 85.37% respectively. Metal chelating capacity was significant as they reduced the concentration of the catalyzing transition metal in lipid peroxidation [28]. The data obtained from Table 4 reveal that extract demonstrate an effective capacity for iron binding, suggesting that its action as antioxidant may be related to its iron binding capacity [20].

CONCLUSION

It can be concluded that the *Euphorbia neriifolia* possesses the significant antioxidant activity compared to other well characterized, standard antioxidant systems *in vitro* and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants, which might be due to the presence of alkaloids, tannins, flavonoids, proanthocynidin and sapogenin. These finding suggest that this plant is a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of ageing and age associated oxidative stress related degenerative diseases such as cancer and various other human ailments.

Further studies are warranted for the isolation and characterization of antioxidant components and also *in vivo* studies are needed for understanding their mechanism of action as an antioxidant better.

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