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Determination of Antioxidant Activity and Phytoconstituent Screening of *Euphorbia heterophylla* Linn

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Authors' contributions

This work was carried out in collaboration between all authors. Author MAA designed and supervised the present study. Author HS performed the experimental study and statistical analysis, reviewed the literature and wrote the manuscript. Author AR co-supervised the present study. Author TR guided during experimental analysis and checked the manuscript. Author MA collected and identified the studies plant and issued its voucher number. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: To carry out qualitative determination of phytochemicals and evaluate antioxidant potential of *Euphorbia heterophylla* Linn.
Place and Duration of Study: Department of Chemistry, Government College University Lahore, Pakistan, between October, 2011 and February, 2012.
Material and Method: The methanolic extract of the plant was dissolved in distilled water and partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol sequentially. The antioxidant potential of all these fractions and remaining aqueous fraction was analyzed by these methods: 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging activity, total antioxidant activity, Ferric Reducing Antioxidant Power (FRAP) assay, Ferric Thiocyanate (FTC) assay while Folin-Ciocalteu colorimetric method was used to analyse total phenolic content. Phytochemical analysis were performed on the plant extracts to detect the presence of secondary metabolites.
Results: Phytochemical screening revealed phenolics and flavonoids in abundance in

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chloroform soluble fraction, ethyl acetate soluble fraction and *n*-butanol soluble fraction. Also the ethyl acetate soluble fraction, *n*-butanol soluble fraction and remaining aqueous fraction contained saponins and sugars. Terpenoids were detected in all other fractions except the aqueous fraction. Alkaloids were determined in ethyl acetate and *n*-butanol soluble fraction only while tannins and cardiac glycosides were present in *n*-butanol soluble fraction and ethyl acetate soluble fraction respectively. Antioxidant assays revealed that Ethyl acetate soluble fraction exhibited highest percent inhibition of DPPH radical i.e. 80.09±0.87% at a concentration of 120 µg/ml as compared to other fractions. IC_{50} value of ethyl acetate fraction was found to be 36.85±1.8 µg/ml relative to ascorbic acid having IC_{50} value 58.8±0.89 µg/ml. It also showed the highest value of total antioxidant activity i.e. 0.918±0.08 as well as highest FRAP value 200.05±0.4 TE µM/ml, highest amount of total phenolic compounds (190.1±1.21 GAE mg/g) and highest percentage of inhibition of lipid peroxidation (54.23±0.57%). Chloroform soluble fraction showed IC_{50} value of 149.84±1.02, total antioxidant activity 0.739±0.06; FRAP value115.15±0.2 µM/ml, total phenolic content 137.1±1.4 GAE mg/g and 41.31±0.53% percent inhibition of lipid peroxidation. *n*-Butanol soluble fraction showed IC_{50} value of 117.67±0.7, total antioxidant activity 0.532±0.03, FRAP value127.5±0.9 µM/ml, total phenolic content 93.5±0.3 GAE mg/g and 32.15±0.9% percent inhibition of lipid peroxidation. *n*-Hexane soluble fraction showed IC_{50} value of 769.7±1.5, antioxidant activity 0.174±0.07, FRAP value 98.26±0.8 µM/ml, total phenolic content 19.5±1.23 GAE mg/g and 12.09±0.8% percent inhibition of lipid peroxidation. Aqueous fraction showed IC_{50} value of 669.3±1.04, antioxidant activity 0.152±0.041; FRAP value 68.7±0.3 µM/ml, total phenolic content 36.3±0.9 GAE mg/g and 25.01±0.96% percent inhibition of lipid peroxidation.

Conclusion: Ethyl acetate soluble fraction was found to be rich in natural antioxidants and a good source of phytochemicals.

Keywords: Euphorbia heterophylla Linn; total antioxidant activity; phytochemicals; DPPH assay; FRAP value; total phenolics; inhibition of lipid peroxidation (%).

1. INTRODUCTION

The role of free radicals and tissue damage in disease such as atherosclerosis, heart failure, neurodegenerative disorders, aging, cancer, diabetes mellitus, hypertension and several other disorders are becoming increasingly recognized [1]. Reactive oxygen species (ROS), as well as reactive nitrogen species (RNS), are products of normal cell metabolism, environmental stresses, and UV irradiation and they are well recognized for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems [2].

Antioxidants, both exogenous and endogenous whether synthetic or natural can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing many disorders [3]. Number of synthetic antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) have been added to foodstuffs. Although these synthetic antioxidants are efficient and cheap, there are some disadvantages because they are suspected of having some toxic properties. Therefore search for natural antioxidants has received much attention and efforts have been made to identify natural compounds that can act as suitable antioxidants to replace synthetic ones [4]. Currently there is growing interest towards natural antioxidants of herbal resources. Epidemiological and *in vitro* studies on

medicinal plants and vegetables strongly supported this idea that plants are capable of exerting protective effects against oxidative stress in biological systems [3].

The use of plants and their extracts in treatment of diseases dates back to 460-370 BC when Hippocrates practiced the art of healing by the use of plant based drugs [5](Soforowa, 1982). Medicinal plants are of great importance to the health of individuals and communities in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds [6]. The use of plants as spices and herbs indicates that the oxidative and antimicrobial constituents are present in all parts of the plants including tree barks, stalks, leaves, fruits, roots and so on [7].

The Euphorbiaceae family is the 4th largest family of the angiosperms comprising over 300 genera and about 7500 species distributed widely in tropical Africa [8]. The Euphorbiaceae plants are shrubs, trees, herbs or rarely lianas [9]. Generally they have characteristic milky latex [10], sticky sap, co-carcinogenic, severe skin irritant and toxic to live stock and humans [11].

In the family Euphorbiaceae genus Euphorbia is a giant genus of flowering plants consisting of 2000 known species varying from annuals to trees having unique flower structure and contain latex. *Euphorbia hirta* possesses anti-bacterial, anthelmintic, anti-asthmatic, sedative, anti-spasmodic, anti-fertility, anti-fungal and anti-malarial properties. Latex of *E. ingens*, *E. mey*, *E. tirucalli* and *E. triangularis* are possible sources of rubber [12]. *E. nerrifolia* is reported to have analgesic, hepatoprotective, immunostimulant, anti-inflammatory, mild CNS depressant, wound healing and radioprotective characteristics [13]. *E. prostrata* is used as anti-hemorrhoidal, anti-inflammatory, analgesic, hypolipidemic, anti-diabetic, anti-dirroheal, anti-asthmatic and for various skin diseases, also, traditionally as snake bite remedy [14]. *Euphorbia prolifera* is used for the treatment of inflammation and tumors [15].

Euphorbia heterophylla Linn is medicinal plant with the common name “spurge weed”. *Euphorbia heterophylla* leaf is used in traditional medical practices as laxative, anti-gonorrheal, migraine and wart cures. The plant latex has been used as fish poison and insecticide and ordeal poisons [16,17]. The leaves of *E. heterophylla* have been reported to contain quercetin [18] (Falodun and Agbakwuru, 2004). Diterpenoids have also been reported in the root of *E. heterophylla* [19]. The skin irritant, tumor-promoting and anti-tumor/anti-cancer and recently anti-HIV activities of Euphorbia species have also been reported in *E. heterophylla* leaf Linn [20].

The present work was aimed to investigate the phytoconstituents and evaluate the antioxidant potency of *Euphorbia heterophylla* plant, therefore, justifying the use of plant in ethno-medicine for treatment of various ailments.

2. MATERIALS AND METHODS

2.1 Plant Collection

The fresh plant *Euphorbia heterophylla* was collected from Azad Kashmir (Pakistan) in August 2011. The plant was identified by Mr. Muhammad Ajaib (Taxonomist), Department of

Botany, G.C University, Lahore. A voucher specimen (GC.Bot.Herb.580) is kept in the Herbarium of the Botany Department of the same university.

2.2 Extraction and Fractionation

The shade-dried ground whole plant was exhaustively extracted with methanol on the soxhlet apparatus. The extract was evaporated in rotary evaporator (Laborta 4000-efficient Heidolph) at 45ºC under vaccum to yield the residue which was dissolved in distilled water and partitioned with *n*-hexane (400ml x 4), chloroform (400ml x 4), ethyl acetate (400ml x 4), *n*-butanol (400ml x 4) respectively. These organic fractions and remaining aqueous fractions were concentrated separately on rotary evaporator, (*n*-hexane at 35ºC, chloroform at 42ºC, ethyl acetate 45ºC, *n*-butanol at 70ºC and water at 60ºC under vacuum) and the residues thus obtained were used to evaluate their phytoconstituents and *in vitro* antioxidant potential.

2.3 Chemicals and Standards

DPPH (1,1-diphenyl-2-picryl hydrazyl), TPTZ (2,4,6 Tripyridyl-s-triazine), Trolox, Gallic acid, Follin-Ciocalteu's phenol reagent and BHT (Butylated hydroxytoluene) were obtained from Sigma Chemical Company Ltd. (USA) and organic solvents (*n*-hexane, chloroform, ethyl acetate, *n*-butanol), sulphuric acid, sodium phosphate, ammonium molybdate, ferric chloride, ferrous chloride, ceric sulphate, hydrochloric acid, copper sulphate, aluminium chloride, lead acetate, acetic acid, linoleic acid, tween-20, ammonia from Merck (Pvt.) Ltd. (Germany).

2.4 Phytochemical Screening

The phytochemical screening testing for the presence of phytoconstituents was performed using the standard procedures [21,22,23].

2.4.1 Test for alkaloids

For the test of alkaloids the TLC card having spots of the studied samples was sprayed with Draggendroff's reagent. Appearance of orange colour indicated the presence of alkaloids.

2.4.2 Test for terpenoids

Two methods were used to test presence of terpenoids.

- First, Ceric sulphate solution was sprayed on TLC card having spots of samples. TLC card was heated on a TLC heater. Appearance of brown colour indicated the presence of terpenoids.
- Second, to 0.5 g of each of the extract was added 2 ml of chloroform. 3 ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the interface indicated the presence of terpenoids.

2.4.3 Test for saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

2.4.4 Test for tannins

2 ml of sample was taken in test tube and 5 ml of *n*-butanol-HCl solution was added. Mixture was warmed for 1 hour at 95ºC in a water bath. Appearance of red colour indicated the presence of tannins.

2.4.5 Test for sugars

Sample solutions (0.5 g in 5 ml of water) were added to boiling Fehling's solution (A and B) in a test tube. Formation of red precipitates indicated the presence of sugars.

2.4.6 Test for phenolics

Neutral ferric chloride was added to each fraction. Appearance of bluish green colour indicated presence of phenolics.

2.4.7 Test for flavonoids

Four methods were used to test for flavonoids.

- First, dilute ammonia (5 ml) was added to a portion of sample solution in water. Concentrated sulphuric acid (1 ml) was added. A yellow colouration that disappears on standing indicated the presence of flavonoids.
- Second, a few drops of 1% aluminium chloride solution were added to sample solution. A yellow colouration indicated the presence of flavonoids.
- Third, the TLC card having spots of samples was sprayed with Benedict's reagent. Green flourescence in UV light indicated the presence of flavonoids.
- Fourth, the TLC card having spots of sample was sprayed with lead acetate solution. Green flourescence in UV light indicated the presence of flavonoids.

2.4.8 Test for cardiac glycosides (keller-killiyani test)

To 0.5 g of each sample diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout the layer.

2.5 Antioxidant Assays

Following analytical antioxidant assays were performed on all the studied fractions.

2.5.1 DPPH radical scavenging activity

The DPPH radical scavenging activities of various fractions of plant were examined by comparison with that of known antioxidant, ascorbic acid using the reported method [24]. Briefly, various concentrations of the samples (1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 60 µg/ml, 30 µg/ml, 15 µg/ml) were mixed with 3 ml of methanolic solution of DPPH (0.1mM). The mixture was shaken vigorously and allowed to stand at room temperature for one hour. Then absorbance was measured at 517 nm against methanol as a blank in the UV–visible

spectrophotometer (CECIL instruments CE 7200 Cambridge England). Lower absorbance of spectrophotometer indicated higher free radical scavenging activity.

The percent of DPPH decoloration of the samples was calculated according to the formula:

$$\text{Antiradical activity} = A_{control} - A_{sample} / A_{control} \times 100$$

Each sample was assayed in triplicate and mean values were calculated.

2.5.2 Total antioxidant activity by phosphomolybdenum complex method

The total antioxidant activities of various fractions of plant were evaluated by phosphomolybdenum complex formation method [25]. Briefly, 500 µg/ml of each sample was mixed with 4 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in sample vials. The blank solution contained 4 ml of reagent solution. The vials were capped and incubated in water bath at 95ºC for 90 minutes. After the samples had been cooled to room temperature, the absorbance of mixture was measured at 695 nm against blank. The antioxidant activity was expressed relative to that of BHT. All determinations were assayed in triplicate and mean values were calculated.

2.5.3 Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was done according to Benzie and Strain [26] with some modifications. The stock solutions included 300 mM acetate buffer (3.1g $CH_3COONa.3H_2O$ and 16 ml CH_3COOH), pH 3.6, 10 mM Hydrochloric acid and 20 mM Ferric chloride hexahydrate solution. The fresh working solution was prepared by mixing 25ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml $FeCl_3.6H_2O$ solution and then warmed at 37ºC before using. The solution of plant samples 500 µg/mL and that of trolox were formed in methanol. 10 µl of each of sample solution and BHT solution were taken in separate test tubes and 2990 µl of FRAP solution was added in each to make total volume up to 3 ml. The plant samples were allowed to react with FRAP solution in the dark for 30 minutes. Reading of the coloured product [ferrous tripyridyltriazine complex] was then taken at 593 nm by UV–visible spectrophotometer. The FRAP values were determined as micromoles of trolox equivalents per ml of sample by computing with standard calibration curve constructed for different concentrations of trolox. Results were expressed in TE µM/ml.

2.5.4 Total phenolic contents

The total phenolics of various fractions of plant were determined by reported method [27]. The 0.1ml (0.5mg/ml) of sample was combined with 2.8ml of 10% Sodium carbonate and 0.1ml of 2N Folin-Ciocalteu's phenol reagent. After 40 minutes absorbance at 725 nm was measured by UV-visible spectrophotometer. Total phenolic contents were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample using the standard calibration curve constructed for different concentrations of gallic acid. The curve was linear between 50 mg/mL to 500 mg/mL of gallic acid. Results were expressed in GAE mg/g.

2.5.5 Ferric thiocyanate (FTC) assay

The antioxidant activities of various fractions of plant on inhibition of linoleic acid peroxidation were assayed by thiocyanate method [28]. The 0.1ml of each of sample solution (0.5 mg/ml) was mixed with 2.5ml of linoleic acid emulsion (0.02 M, pH 7.0) and 2.0ml of phosphate buffer (0.02 M, pH 7.0). The linoleic acid emulsion was prepared by

mixing 0.28 g of linoleic acid, 0.28 g of Tween-20 as emulsifier and 50.0ml of phosphate buffer. The reaction mixture was incubated for 5 days at 40ºC. The mixture without extract was used as control. The mixture (0.1ml) was taken and mixed with 5.0ml of 75% ethanol, 0.1ml of 30% ammonium thiocyanate and 0.1ml of 20 mM ferrous chloride in 3.5% HCl and allowed to stand at room temperature. Precisely 3 minutes after addition of ferrous chloride to the reaction mixture, absorbance was recorded at 500nm. The antioxidant activity was expressed as percentage inhibition of peroxidation (IP %).

[IP% = {1-(abs. of sample) / (abs. of control)} × 100].

The antioxidant activity of BHT was assayed for comparison as reference standard.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Investigation

The present study carried out on the *E. heterophylla* Linn plant samples revealed the presence of medicinally active constituents (Table 1). It was observed from the preliminary phytochemical investigation that phenolics and flavonoids were present in chloroform soluble fraction, ethyl acetate soluble fraction and *n*-butanol soluble fraction while *n*-hexane soluble fraction and remaining aqueous fraction showed absence of these compounds. Ethyl acetate soluble fraction contained phenolics and flavonoids in more amounts as compared to other fractions. Ethyl acetate soluble fraction, *n*-butanol soluble fraction and remaining aqueous fraction contained saponins and sugars while *n*-hexane soluble fraction and chloroform soluble fraction showed absence of these compounds. Terpenoids were detected in all fractions except the aqueous fraction. Alkaloids were present in ethyl acetate soluble fraction and *n*-butanol soluble fraction while absent in all other fractions. Tannins were only present in *n*-butanol soluble fraction. Cardiac glycosides were only present in ethyl acetate soluble fraction. The presence of these phytoconstitents in *Euphorbia heterophylla* plant also confirmed the reports of James et al.,[29] who carried phytochemical analysis on leaf extract of the plant. Also the reported phytoconstituents of *E. hirta* include triterpenoids, sterols, alkaloids, glycosides, flavonoids, tannins, phenols, choline and shikmic acid [12]. *E. nerrifola* predominantly contains sugar, tannins, flavonoids, alkaloids and triterpenoidal saponins [13]. Preliminary phytochemical studies revealed the presence of flavonoids, tannins, glycosides and saponins in the alcoholic extract of the *E. prostrata* [14]. Phytochemical analysis of the crude extracts of the *E. balsamifera* revealed the presence of tannins, saponins, steroid, terpenoid, flavonoids, cardic glycosides and balsam (gum) [30].

Table 1. Phytochemical constituents of various fractions of *Euphorbia heterophylla* Linn

Test	*n*-Hexane soluble fraction	Chloroform soluble fraction	Ethyl acetate soluble fraction	*n*-Butanol soluble fraction	Remaining aqueous fraction
Alkaloids	-	-	+	+	-
Terpenoids	++	++	+++	+++	-
Saponins	-	-	+	+	+
Tannins	-	-	-	+	-
Sugars	-	-	+	++	+++
Phenolics	-	++	+++	+	-
Flavonoids	-	+	++	+	-
Cardiac glycosides	-	-	+	-	-

'+' represents presence and '–' represents absence.

3.2 DPPH Radical Scavenging Activity

Free radical scavenging is one of the known mechanisms by which antioxidants inhibit cellular damage. The DPPH free radical scavenging method is colorimetric assay and can be used to evaluate the radical scavenging capacity of specific compounds or extract in a short time [31]. In the radical form, the molecule of DPPH has an absorbance at 517nm, which will disappear after the acceptance of an electron or hydrogen radical from an antioxidant in the solution to become a stable diamagnetic molecule [32]. Besides, DPPH radical has the advantage of being unaffected by certain side reactions of polyphenols, such as metal ion chelation and enzyme inhibition. It is reported that the decrease in the absorbance of DPPH radical caused by phenolic compound is due to the reaction between antioxidant molecules and radicals, resulting in the scavenging of the radical by hydrogen donation and is visualized as a discoloration from purple to yellow [33]. In this present study, different concentrations of extracts were used. The relationship between the scavenging activity and concentrations are shown in Table 2.

Table 2. Free radical scavenging activity of various fractions of *Euphorbia heterophylla* Linn. using 1, 1-Diphenyl-2-picryl hydrazyl radical (DPPH)

Sr.No.	Sample	Conc.(µg / mL)	% Scavening of DPPH radical ± S.E.M[a)]
1	*n*-Hexane soluble fraction	1000	57.23±1.48*
		500	44.16±0.44
		250	27.99±0.22
		120	22.86±0.34
2	Chloroform soluble fraction	250	69.25±1.2*
		120	53.34±1.05*
		60	27.99±0.79
		30	16.99±1.01
3	Ethyl acetate soluble fraction	120	80.09±0.87*
		60	63.29±0.34*
		30	51.45±0.76*
		15	35.5±0.23
4	*n*-Butanol soluble fraction	120	53.8±0.89*
		60	16.32±0.56
		30	5.75±1.4
		15	4.82±0.87
5	Remaining aqueous fraction	1000	72.31±0.98*
		500	31.88±1.12
		250	13.21±0.8
		120	11.35±0.34
6	Ascorbic acid[b)]	125	79.14± 0.93
		60	58.09 ±0.86
		30	29.97± 0.55

[a)]standard mean error of three assays.
[b)]a reference standard antioxidant.
**p< 0.05 when compared with negative control i.e. blank/solvent (p<0.05 is taken as significant).*

It can be clearly seen that the scavenging activity of *Euphorbia heterophylla* extracts were concentration-dependent. With the increase of the concentration of the samples, the scavenging activity of the extracts increased accordingly. The various concentrations of ethyl

acetate soluble fraction exhibited highest percent inhibition of DPPH radical as compared to other fractions. It showed 80.09 ± 0.87% inhibition of DPPH radical at the concentration of 120 µg/ml. The various concentrations of the fractions which showed percent inhibition greater than 50% were found to be significant ($p < 0.05$) when compared with negative control i.e. blank.

IC_{50} is defined as the concentration of the extracts to quench 50% of DPPH in the solution under the chosen experimental conditions and IC_{50} values given in Table 3. IC_{50} value is inversely related to the activity as it is the measure of inhibitory concentration and a lower value would reflect greater antioxidant activity of the fraction. Ethyl acetate soluble fraction exhibited lowest IC_{50} 36.85±1.8µg/ml compared to other studied fractions. Chloroform soluble fraction, *n*-hexane soluble fraction, *n*-butanol soluble fraction and remaining aqueous fraction showed IC_{50} value 149.84±1.02, 769.7±1.5, 117.67±0.7, 669.3±1.04 relative to Ascorbic acid having IC_{50} of 58.8±0.89 µg/ml. The IC_{50} values of chloroform soluble fraction, ethyl acetate soluble fraction and *n*-butanol soluble fraction were found to be significant ($p < 0.05$) while that of remaining aqueous fraction was found to be non significant ($p > 0.05$) when compared with ascorbic acid, a reference standard.

The extract of *Euphorbia neriifolia* possess efficient scavenging character when compared with the standards and 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 [34].

3.3 Total Antioxidant Activity by Phosphomolybdenum Method

The total antioxidant capacity of all fractions was measured spectrophotometrically by the phosphomolybdenum method, which is based on the reduction of Mo(VI) to Mo(V) by the sample analyte and the subsequent formation of green phosphate/Mo(V) compounds with a maximum absorption at 695 nm [35]. Electron transfer occurs in this assay which depends upon the structure of the antioxidant [25]. The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, tocopherols and carotenoids. The total antioxidant activities of these fractions were compared with the standard antioxidant BHT and the results shown in Table 3. The results indicated that ethyl acetate soluble fraction had highest total antioxidant activity (0.918±0.08). The antioxidant activity of the various plant fractions decreased in the following order: chloroform soluble fraction (0.739±0.06) > *n*-butanol soluble fraction (0.532±0.03) > *n*-hexane soluble fraction (0.174±0.07) > aqueous fraction (0.152±0.041). BHT showed total antioxidant activity 0.925±0.08. The total antioxidant activity shown by chloroform soluble fraction, ethyl acetate soluble fraction and *n*-butanol soluble fraction was found to be significant ($p < 0.05$) while that of *n*-hexane and aqueous fraction were found to be non significant ($p > 0.05$) when compared with BHT.

Also the extract of *E. neriifolia* was found to have higher activity, as compared to the standard (gallic acid). It was revealed that the antioxidant activity of the extract exhibited increasing trend with the increasing concentration of the plant extract [36].

3.4 Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing capacity of compounds may serve as a significant indicator of their potential antioxidant activity. The ferric reducing antioxidant power (FRAP) assay measures the reducing ability of antioxidants against oxidative effects of reactive oxygen species. Electron

donating antioxidants can be described as reductants and inactivation of oxidants by reductants can be described as redox reactions. This assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of tripyridyltriazine [TPTZ] forming an intense blue Fe^{2+}-TPTZ complex with an absorbance maximum at 593 nm [26]. Increasing absorbance indicates an increase in reductive ability. From the results it was revealed that ethyl acetate soluble fraction showed highest FRAP value of 200.05±0.4 μM/ml. Chloroform soluble fraction and *n*-butanol soluble fraction also showed good FRAP values 115.15±0.2 μM/ml, 127.5±0.9 μM/ml respectively while *n*-hexane soluble fraction and remaining aqueous fraction showed very less FRAP values i.e. 98.26±0.8 μM/ml, 68.7±0.3 μM/ml respectively. High FRAP values obtained for polar fractions may be ascribed partially to the phenolic and flavonoid contents. The FRAP values of chloroform soluble fraction, ethyl acetate soluble fraction, and *n*-butanol soluble fraction were found to be significant ($p < 0.05$) while that of *n*-hexane soluble fraction and remaining aqueous fraction were found to be non significant ($p > 0.05$) when compared with blank.

The FRAP values of *E. nerrifolia* extract was found to be significantly higher as compared to the standards i.e. quercetin and BHT and thus the extract of *E. nerrifolia* act as free radical scavenger, capable of transforming reactive free radical species into stable non radical products [36].

3.5 Total Phenolic Content

The therapeutic effects derived from several medicinal plants have been attributed to the presence of phenolic compounds such as flavonoids, phenolic acid, proanthocyanidins, diterpenes and tannins [37]. These compounds exhibit antioxidant activity by inactivating lipid free radicals or by preventing the decomposition of hydroperoxides into free radicals [38]. Phenolic compounds are known to inhibit various types of oxidizing enzymes. These potential mechanisms make the diverse group of phenolic compounds an interesting target in the search for beneficial phytochemicals [39]. Phenols are one of the major groups of non-essential inhibition of atherosclerosis that have been associated with the inhibition of atherosclerosis and cancer, as well as for age-related degenerative brain disorders [31]. Phenolics, a well-known group of plant secondary metabolites, are prominent free radical scavengers and also responsible for exhibiting multiple medicinal and physiological functions in animals as well as in plants [40]. Table 3 shows the total phenol content (TPC) expressed as gallic acid equivalents (GAE) achieved by FC method. Moreover, it is well known that plant phenolics are highly effective free radical scavengers and antioxidants, and the activity is derived largely from the phenolic and polyphenolic compounds. Therefore, the investigation on the antioxidant activity of *E. Heterophylla* extracts was of great importance. The ethyl acetate soluble fraction showed the highest amount of total phenolic compounds 190.1±1.21 GAE mg/g while that of chloroform soluble fraction was found to be 137.1±1.4 GAE mg/g. The total phenolic contents of *n*-hexane soluble fraction, *n*-butanol soluble fraction and remaining aqueous fraction were found to be 19.5±1.23, 93.5±0.3, 36.3±0.9 GAE mg/g respectively. The results for total phenolic contents of chloroform soluble fraction, ethyl acetate soluble fraction, and *n*-butanol soluble fraction were found to be significant ($p < 0.05$) while that of *n*-hexane soluble fraction and remaining aqueous fraction were found to be non significant ($p > 0.05$) when compared with blank.

The total phenolic content of *E. nerrifolia* extract was found to be significantly higher as compared to the reference standard [34].

3.6 Ferric Thiocyanate (FTC) Assay

It has been reported that the lipid peroxidation is one of the causes of the occurrence of cardiovascular disease and cancer. Lipid peroxidation contains a series of free radical mediated chain reaction processes and is also associated with several types of biological damages [41]. Therefore, the inhibition of peroxidation by the plant extracts indicates its antioxidant potency. Hydrogen donating antioxidants can react with lipid peroxyl radicals and break the cycle of generation of new radicals. The ferric thiocyanate method is used to measure the amount of peroxide at the beginning of lipid peroxidation, in which peroxide will react with ferrous chloride and form ferric ions. Ferric ions will then unite with ammonium thiocyanate and produce ferric thiocyanate, a reddish pigment [42]. The fractions which showed greater values of percent inhibition of lipid peroxidation might contain primary antioxidant compounds which are able to react aggressively with free radicals, particularly hydroxyl radicals thereby terminating the radical-chained reaction and retarding the formation of hydroperoxides [43]. In this study, the peroxidation inhibition activity of the *E. heterophylla* extracts was measured and shown in Table 3. The data revealed, inhibition percentage of lipid peroxidation was high in the order of ethyl acetate soluble fraction (54.23±0.57%) > chloroform soluble fraction (41.31±0.53%) > *n*-butanol (32.15±0.9%) > aqueous fraction (25.01±0.96%) > *n*-Hexane soluble fraction (12.09±0.8%). The inhibition of lipid peroxidation by BHT (standard) was 62.48±1.07%. The data revealed that the ethyl acetate fraction and chloroform soluble fractions showed a good inhibition of lipid peroxidation formed during linoleic acid system. The high peroxidation scavenging effect may be due to the high contents of phenolic compounds or radical scavengers involved in the extracts [44] which can terminate the peroxidation chain reactions easily [45] and quench reactive oxygen or nitrogen species, thereby inhibiting the oxidation of lipids and other biological molecules [46]. The results for percent inhibition of lipid peroxidation of chloroform soluble fraction, ethyl acetate soluble fraction and *n*-butanol soluble fraction were found to be significant ($p < 0.05$) while that of *n*-hexane soluble fraction and aqueous fraction were found to be non significant ($p > 0.05$) when compared with BHT.

The *E. neriifolia* extract also exhibit good effect in inhibiting linoleic acid oxidation compared to control (BHT). The antioxidant activities also increased with increasing concentration of the *E. neriifolia* extract. The phenolic compounds present donate hydrogen and terminate the free radical reaction chain by changing it to the stable compounds [36].

3.7 Statistical Analysis

All the measurements were done in triplicate and statistical analysis was performed by Statistical software. All the data were expressed as ± S.E.M. Statistical analysis were determined using one way analysis of variance (ANNOVA) followed by post-hoc Tukey's test.

Table 3. IC_{50}, total antioxidant activity, FRAP values, total phenolics and lipid peroxidation of different fractions of *Euphorbia heterophylla* Linn

Sr. No	Sample	IC_{50} (µg/mL)	Total antioxidant activity	FRAP value TE (µM/ml)	Total phenolics (GAE mg/g)	Inhibition of lipid peroxidation (%)
1	*n*-Hexane soluble fraction	769.7±1.5	0.174±0.07	98.26±0.8	19.5±1.23	12.09±0.8
2	Chloroform soluble fraction	149.84±1.02**	0.739±0.06**	115.15±0.2*	137.1±1.4*	41.31±0.53**
3	Ethyl acetate soluble fraction	36.85±1.8**	0.918±0.08**	200.05±0.4*	190.1±1.21*	54.23±0.57**
4	*n*-Butanol soluble fraction	117.67±0.7**	0.532±0.03**	127.5±0.9*	93.5±0.3*	32.15±0.9**
5	Remaining aqueous fraction	669.3±1.04	0.152±0.041	68.7±0.3	36.3±0.9	25.01±0.96
6	Ascorbic acid[a)]	58.8 ± 0.89	-	-	-	-
6	BHT [a)]	-	0.925±0.08	-	-	62.48±1.07
7	Blank	-		24.11 ± 0.95	14.96±0.56	-

All results are presented as mean ± standard mean error of three assays.

[a)] Standard antioxidants.

**p< 0.05 when compared with negative control i.e. blank/solvent (p<0.05 is taken as significant).*

***p < 0.05 when compared with reference standards (BHT/Ascorbic acid).*

4. CONCLUSION

The present results confirmed that different fractions especially ethyl acetate soluble fraction, also, chloroform soluble fraction and *n*-butanol soluble fraction of *Euphorbia heterophylla* Linn plant are rich in phytochemicals and highly valueable source of natural antioxidants and free radical scavengers. These extracts can increase the shelf life of food products, as well as, they can be used against damaging effects of free radicals and can inhibit degenerative disorders and carcinogenesis and delay aging.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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