Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 8, 2014

Original Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIOXIDANT POTENTIAL OF *EUPHORBIA HETEROPHYLLA* L

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Received: 17 Jun 2014 Revised and Accepted: 05 Aug 2014

ABSTRACT

Objectives: The present study is to evaluate the preliminary phytochemical screening and *in vitro* antioxidant potential of *Euphorbia heterophylla* L.

Methods: *In vitro* free radical scavenging activity of the chosen plant was evaluated by the methods such as DPPH (1, 1-Diphenyl -2-picrylhydrazyl), reducing power assay, Nitric oxide radical scavenging assay, Hydrogen peroxide scavenging assay (H_2O_2) and superoxide scavenging assay. Preliminary phytochemical screening and quantification of total phenol, ascorbic acid and organic compounds were also studied.

Results: The results showed that the aqueous extract of *Euphorbia heterophylla* L. has potent antioxidant activities as evidenced by the IC_{50} values obtained in DPPH (141.11±4.23µg/ml), Hydrogen peroxide (510.03±2.45µg/ml), Superoxide radical (596.94±1.98µg/ml), reducing power (258.08±3.21µg/ml), Nitric oxide (596.94±6.02µg/ml) scavenging assays. Preliminary phytochemical screening of the aqueous extract of the plant shows the presence of various secondary metabolites such as alkaloid, flavonoid, tannin, saponin, sterol, quinone, lignin and coumarin. The carbohydrate (10.29 mg/g), proteins (7.43 mg/g), were present in measurable amount and was considered to be responsible for the nutritional facts and the phenol (3.26mg/g) and ascorbic acid (1.14%) content attribute to its antioxidant activities.

Conclusion: The results of the present study concluded that the aqueous extract of *Euphorbia heterophylla* L. posses the antioxidant activity.

Keywords: *Euphorbia heterophylla* L, *In vitro* antioxidant, Phytochemicals.

INTRODUCTION

Oxidants are both molecular and free radical in nature. Free radicals are produced during oxidation/reduction reaction involving transfer of one electron, or when a covalent bond is broken and one electron from each pair remains with each group. Due to the presence of unpaired electron(s), free radicals are considerably more reactive and participate in a number of physiological and patho-physiological functions [1].

Most degenerative diseases that afflict humanity have their origin in deleterious free radicals was evidenced. This disease includes atherosclerosis, cancer, inflammatory joint disease, asthma, diabetes, senile dementia and degenerative eye disease. The process of biological ageing might be due to the damage of cells by free radicals. These free radicals cause lipid peroxidation in cell membranes, and inactivate membrane-bound enzymes [2].

The antioxidant or radical scavenging property is more important because of its potential to provide health protection against reactive oxygen species and free radicals, which are the main cause of many diseases [3]. These antioxidants provide protection to living organisms from damage caused by uncontrolled production of ROS and concomitant lipid peroxidation, protein damage and DNA strand breaking [4]. Over the last few decades, there has been an increased interest in the research globally to identify and validate about the antioxidant compounds that are pharmacologically potent with minimal side effects.

Plants have developed an array of defense strategies (antioxidant system) to cope up with oxidative stress. Plants are rich sources for natural antioxidants, the best known are tocopherols, flavonoids, vitamin C and other phenolic compounds [5]. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide of lipid hydroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [6]. There are number of plants are reported for their antioxidant activity because of the presence of constituents such as phenolic acids, flavonoids, tannins, coumarins etc., [7] The antioxidant activity of plant and its compound can be assessed using *in vitro* methods which involve the prevention of oxidation and its damage by inhibiting or quenching the free radicals and reactive oxygen species and can be compared with synthetic antioxidants [8].

Euphorbia heterophylla L. is branched shrub belonging to the family *Euphorbiaceae* and are widely distributed in India, South Asian countries, Africa, Mexico, Thailand. It is an ornamental plant and common names include Paalperuki in tamil, Mexican fireplant, poinsettia etc., This plant is reported to have wound healing activity [9], anti-inflammatory [10], antimicrobial and anticancer activity [11].

In traditional medicine it is used to treat constipation, bronchitis, asthma, laxative and as lactogenic agent. In the present studies attempt has been made to evaluate the *in vitro* free radical scavenging activity of aqueous extract of the leaves of *Euphorbia heterophylla* L.

MATERIALS AND METHODS

Plant source selected for the present study is *Euphorbia heterophylla* L. The leaves of the plant was collected, identified with the help of The Flora of Presidency of Madras [12] and authenticated with the help of herbarium specimen deposited at RAPINAT herbarium of St. Joseph's College, Trichy, Tamilnadu, India. The qualitative determination of phytochemical constituents present in the aqueous extract of *Euphorbia heterophylla* L was carried out as per the textual procedure [13]. The plant samples were prepared for the determination of micro and macronutrients using the methods given in shah *et al* [14].

Preparation of aqueous extract

Fresh plant material was shade dried and powdered coarsely using electric blender. The plant powder 200 gm of *Euphorbia heterophylla* L. was taken and extracted with water. To one part of the plant powder six parts of water was added, boiled and reduced to one third and filtrated. Then the filterate was evaporated to dryness. Paste form of the extract obtained was subjected to *in-vitro* assays.

In-vitro free radical scavenging assays

DPPH radical scavenging assay

The antioxidant activity of aqueous extract of *Euphorbia heterophylla* L was evaluated by DPPH scavenging assay [15]. Antioxidants react with DPPH, a stable free radical that was reduced to DPPH: H form. The degree of discoloration indicates the scavenging potential by donating hydrogen ion of the antioxidant compound present in the plant. Briefly 100 µl of various concentrations of plant extract was added to a 100 µl of 200 mM ethanol solution of DPPH. After 30 minutes incubation at room temperature, the absorbance was read at 517 nm. Ascorbic acid was taken as standard reference. The percentage scavenging activity of plant extract on DPPH free radical was calculated by

DPPH Scavenging Activity (%) = A Control - A Test / A Control × 100

A Test is the absorbance of test solution (plant extract). A Control is absorbance of control.

Reducing power assay

The reducing power of aqueous extract of *Euphorbia heterophylla* L was determined using the method described [16]. A serial dilution of the plant extract was taken (100, 200, 300, 400 and 500 µg/mL) add 1 ml of 1% ferrocyanate in 0.2 M phosphate buffer pH, 6.6. The mixture was incubated at 50 ºC for 20 minutes. Then 10% trichloroacetic acid (TCA, 2.5 mL) was added to a portion of this mixture (5 mL) and centrifuged at 3,000 g for 10 minutes. The supernatant was separated and mixed with distilled water (2.5 mL) containing 1% ferric chloride (0.5 mL). The absorbance of this mixture was measured at 700 nm. The intensity in absorbance could be the measurement of antioxidant activity of the plant extract.

Hydrogen peroxide scavenging capacity

The ability of the aqueous extract of *Euphorbia heterophylla* L (leaves) to scavenge hydrogen peroxide was determined according to the method of Ruch et al [17]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts of different concentration (200 µg/mL to 1000 µg/mL) in distilled water were added to the hydrogen peroxide solution (0.6 mL) and incubated for 10 minutes. Absorbance of hydrogen peroxide at 230 nm was determined against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging capacity was calculated.

Nitric oxide radical scavenging assay

The nitric oxide scavenging activity was determined using Griss reagent [18]. Various concentrations (200 µg/mL to 1000 µg/mL) of aqueous extract *Euphorbia heterophylla* L (leaves) was taken in different test-tubes. 0.5 ml of sodium nitroprusside (5 mM) in phosphate buffer was added to each test tube to make volume up to 3 ml with phosphate buffer. Solutions were incubated at 25ºC for 3hr. Thereafter, 0.5 ml of Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 3% phosphoric acid) was added to each test tube. The absorbance was measured immediately at 546 nm and percentage of scavenging activity was measured with reference to ascorbic acid as standard.

Superoxide radical scavenging assay

The scavenging capacity for superoxide radical was assessed using Alkaline DMSO as a super oxide generating system [19]. To the different concentrations of the plant extracts, 1 ml of alkaline DMSO and 0.2 ml of NBT 20 mM in phosphate buffer pH 7.4 was added.. The reaction mixture was illuminated for 30 mins and absorbance was measured at 560 nm. (Govindarajan, 2003).

RESULTS AND DISCUSSION

The Phytochemical screening of plants has importance in identifying new source of therapeuticaly valuable compounds having medicinal significance. A number of plants have been chemially investigated [20]. The study also evaluates the presence of phytochemical constituents. Preliminary phytochemical screening showed the presence of alkaloids, flavonoids, tannins, saponin glycosides, and phenolic compounds. Presence of these secondary metabolites may attributes to its antioxidant potentials.

Quantitative organic analysis

The amount of macronutrients present in the aqueous extract of *Euphorbia heterophylla* L. was tabulated in the Table 1. The plant contain moderate amount of carbohydrates (10.29 mg/g), protein (7.43 mg/g). Total phenolic content was found to be (3.26mg/g). Phenolics are antioxidants with redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. They also have metal chelation properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action [21]. The phenolic compounds have important role in counteracting reactive oxygen species (ROS) and thus minimizing molecular damage. The plant phenolics can act as singlet oxygen quenchers and scavenge free radicals. The content of ascorbic acid present was 1.14% it may boost the antioxidant potentials. In cell and tissues the ascorbic acid scavenges the free radicals, it act as a chelating agent and it plays an important role in defense mechanisms [22].

Table 1: Quantification of primary metabolites

S. No.	Parameters	Values(mg/g)
1.	Total Carbohydrate	10.29
2.	Total Proteins	7.43
3.	Total Fat	3.5

Table 2: Determination of Phenolic and ascorbic acid content

S. No.	Parameters	Values
1.	Total Phenolic content(mg/g)	8.26
2.	Ascorbic content (%)	1.14

In vitro Antioxidant activity

DPPH radical scavenging assay

The DPPH assay showed the ability of the test drug to act as a free radical scavenger and it is extensively used for screening antioxidants from vegetables, fruits and extracts [23]. DPPH assay method is based on the ability of 1, 1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. DPPH is a protonated radical, with absorbance maximum at 517 nm, and decreases with the scavenging of the proton radical. This property has been widely used to evaluate the free radical scavenging effect of natural antioxidants [24]. The free radicals in DPPH can be neutralized by the antioxidants present in plants extract by transferring either their electrons or by hydrogen atoms to DPPH thereby changing the color from purple to yellow-colored diphenyl picrylhydrazine [25]. The aqueous extract of *Euphorbia heterophylla* L. showed the considerable radical scavenging activity in a concentration dependant manner upto 250 µg/ml and compared with standard ascorbic acid given in the **Figure - 1**. The reference standard ascorbic acid also showed a significant radical scavenging potential at a concentration of 30µg/ml. The % inhibition of DPPH was strongly dependant on concentration of the plant extract [26]. The mean IC_{50} value of the plant extract was found to be 141.11µg/ml. The lower IC_{50} indicates higher antioxidant activity [27].

Reducing power assay

The antioxidant potential of the plant was indicated by reducing power assay. The presence of antioxidants in the plant extracts results in the reduction of the Fe3+/ferricyanide complex into its ferrous form which is measured by the intensity of the resultant Prussian blue color complex. The higher absorbance at high concentration indicates the strong reducing capacity. The reducing power of aqueous extract of *Euphorbia hetrophylla* L was very potent and the reducing power of plant was increased with increase in concentration which is showed in the **Figure-2**. The extract

showed maximum activity of **92.12** % at its maximum concentration (500μg/ml) and compared to standard ascorbic acid. The IC_{50} value for reducing power was found to be 258.08μg/ml. Electron donating capacity of the plant extracts convert free radicals into more stable products and terminate the radical chain reaction [28]. Presence of the polyphenoic compounds in the plant extracts is responsible for the reducing power capacity [29].

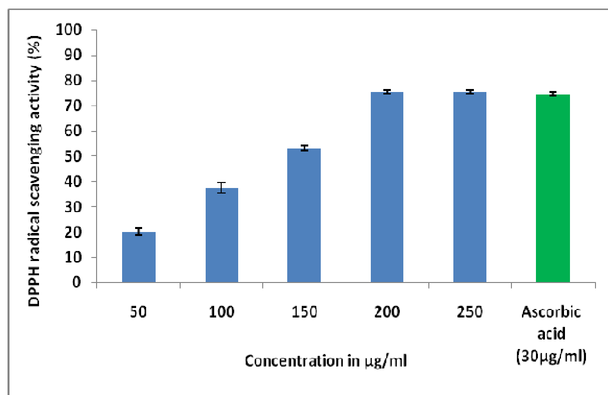

Fig. 1: DPPH radical scavenging activity of *Euphorbia heterophylla* L The Data was represented as Mean±SD. Experiment was done in triplicates n=3.

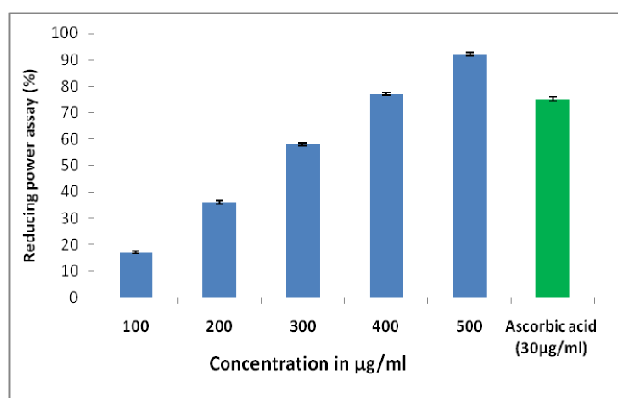

Fig. 2: Reducing power ability of *Euphorbia heterophylla* L The Data was represented as Mean±SD. Experiment was done in triplicates n=3.

Hydrogen peroxide scavenging assay

The hydrogen peroxide scavenginng assay of the aqueous plant extract was shown in the **Figure – 3**. Hydrogen peroxide is a highly important reactive oxygen species due to its ability to penetrate biological membranes. However, it may be toxic if converted to OH in the cell if it reacts with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals and this may be the origin of many of its toxic effects. Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups [30, 31]. In the present study, the extracts were capable of scavenging H_2O_2 dose dependently. The percentage of hydrogen peroxide scavenging activity of aqueous leaf extracts of *Euphorbia heterophylla* L was 83.26% at a concentration of 100μg/ml. and compared to the standard ascorbic acid (89.80%). The mean IC_{50} value was found to be 510μg/ml.

Nitric oxide radical scavenging assay

Nitric Oxide is known to play a crucial role in various physiological processes such as smooth muscle relaxation, neuronal signaling, and inhibition of platelet aggregation and regulation of cell-mediated toxicity. It is a diffusible free radical, which plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation, antimicrobial and antitumor activities [32]. Nitric oxide is a very unstable species and reacts with oxygen molecule produce stable nitrate and nitrite, which can be estimated by using Griess reagent.

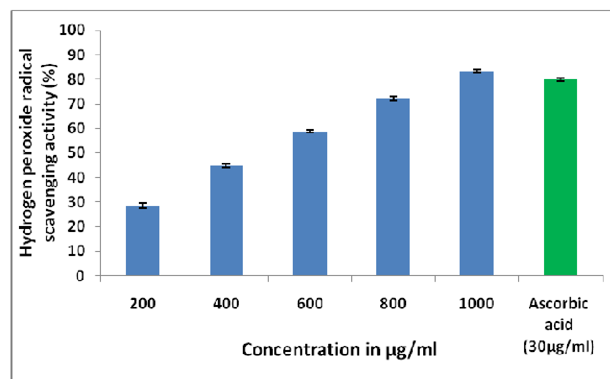

Fig. 3: Hydrogen peroxide radical scavenging activity of *Euphorbia heterophylla* L. The Data was represented as Mean±SD. Experiment was done in triplicates n=3.

In the presence of a scavenging test compound, the amount of nitrous acid will decrease which can be measured at 546 nm. Aqueous extract of *Euphorbia heterophylla* L results in increased nitric oxide antiradical activity with increasing concentration (**Figure-4**). The maximum scavenging activity **78.77** % was found at the concentration of 1000μg/ml. The mean IC_{50} value was found to be (596.94μg/ml). This percentage inhibition displayed by the plant extract showed that plant extract is a potent scavenger of nitric oxide in the dose dependant manner.

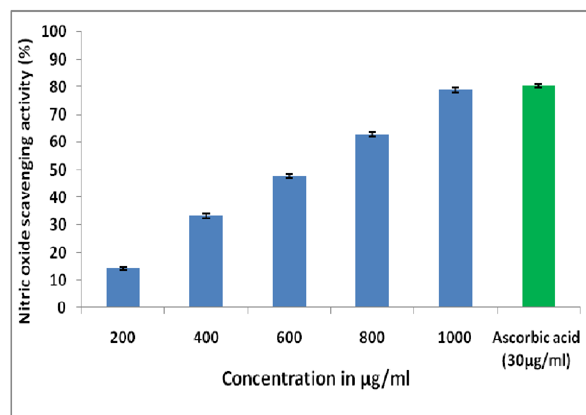

Fig. 4: Nitric oxide scavenging activity of *Euphorbia heterophylla* L. The Data was represented as Mean±SD. Experiment was done in triplicates n=3.

Superoxide radical scavenging assay

Superoxide radical is highly toxic species, which is generated by numerous biological and photochemical reactions [33]. Superoxide dismutase is an important antioxidant defense enzyme that scavenges the superoxide anion by converting to hydrogen peroxide and decreases the toxicity of this radical [34]. In the PMS-NADH-NBT system, superoxide anion derived from dissolved oxygen, by PMS-NADH coupling reaction, reduces NBT (yellow dye) to blue-colored product called formazon. Drugs possessing superoxide scavenging activity decreases the reduction of NBT, which is a measure of superoxide anion scavenging activity that is indicated by reduction in absorbance at 560 nm. Superoxide radical scavenging activities of *Euphorbia heterophylla* L showed maximum activity (97.01 %) at high concentration of 100μg/ml, where as the low activity (22.28%) was presented in the **Fig-5.** The mean IC_{50} value for superoxide

radical was found to be 425.8μg/ml and it was compared with standard ascorbic acid. The superoxide anion scavenging activity of the extract of *Euphorbia heterophylla* L may be due to the high phenolic contents and flavonoids [35].

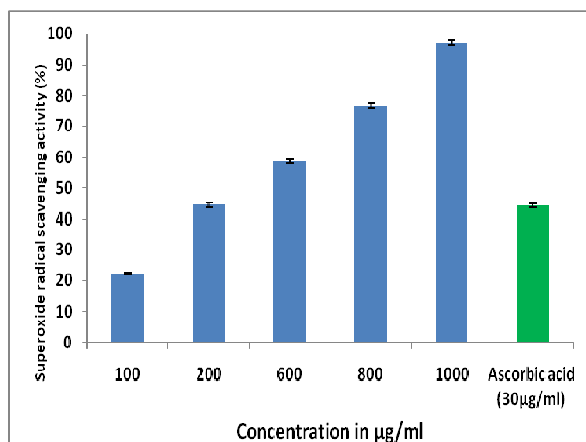

Fig. 5: Superoxide radical scavenging activity of *Euphorbia heterophylla* L. The Data was represented as Mean±SD. Experiment was done in triplicates n=3.

CONCLUSION

In this investigation the invitro antioxidant activity of the aqueous extract of aerial parts of *Euphorbia heterophylla* L was determined. The DPPH free radical scavenging activity, reducing power assay, Nitric oxide scavenging activity, superoxide anion scavenging activity and hydrogen peroxide radical scavenging assay exhibited significant scavenging activity. This may be due to the presence of flavonoids, alkaloids, phenols and ascorbic acid which was confirmed by preliminary phytochemical screening and quantitative analysis. The results suggest that aqueous extract of *Euphorbia heterophylla* L contains antioxidant effect and it can be considered for the prevention and treatment for many diseases.

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