Research Article

In Vitro Antioxidant Activity of *Euphorbia geniculata* Leaves

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ABSTRACT

Many oxidative stress related diseases are as a result of accumulation of free radicals in the body. Free radicals have been implicated in the causation of several problems like asthma, cancer, cardiovascular disease, cataract, diabetes, gastrointestinal inflammatory disease, liver disease, muscular degeneration and other inflammatory process. Evidence suggests that compounds especially from natural sources are capable of providing protection against free radicals. In view of the above facts, this study was designed to evaluate *in vitro* antioxidant activities of methanol and chloroform extract of *Euphorbia geniculata* leaves. *Euphorbia geniculata* exhibited its scavenging effect in concentration dependent manner on 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), hydroxyl radical, nitric oxide, superoxide and hydrogen peroxide. The extract was also effective in preventing lipid peroxidation. These *in vitro* assays indicate that this plant extract is a potential source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords: *Euphorbia geniculata,* antioxidant, DPPH, ABTS, lipid peroxidation.

INTRODUCTION

Since ancient times, the medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities. A rich heritage of knowledge to preventive and curative medicines was available in ancient scholastic works included in the Atharva veda, Charaka, Sushruta etc. Now a days 80% people (WHO estimated) from all over world are interested towards traditional medicines[1]. Flavonoids and phenolic compounds widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, antimicrobial, anti-adhesion, anti-carcinogenic properties while others may be involved in boosting the immune system, or aiding general metabolism

Oxidative stress results from generation of oxygen free radicals, hydrogen peroxide, hydroxyl radical, hydroperoxide, dioxygen and nitric oxide, collectively termed as reactive oxygen species (ROS). In living organisms, the reactive oxygen species (ROS) and reactive nitrogen species (RNS) are known to cause damage to lipids, proteins, enzymes, and nucleic acids leading to cell or tissue injury implicated in the processes of aging as well as in wide range of degenerative diseases including inflammation, cardiovascular diseases, cancer, atherosclerosis, diabetes, liver injury, Alzheimer, Parkinson, and coronary heart pathologies[2]. Recently, there has been growing scientific interest to find naturally occurring antioxidants because of established carcinogenicity of used synthetic antioxidants. It has been estimated that the average person has around 10,000-20,000 free radicals attacking each body cell each day[3]. Free radicals perform many critical functions in our bodies in controlling the flow of blood through our arteries, to fight infection, to keep our brain alert, in focus and they are responsible for turning on and off of genes.

Natural antioxidants present in the plants scavenge harmful free radicals from our body. As antioxidants have been reported to prevent oxidative damage caused by free radical, it can interfere with the oxidation process by reacting with free radicals, chelating and catalytic metals and also by acting as oxygen

scavengers[4,5]. Novel natural antioxidants from some plants have been extensively studied in the past few years for their antioxidant and radical scavenging properties. It is possible to reduce the risk of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants[6]. Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used in foods have side effect and are carcinogenic[7]. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins[8].

Euphorbia geniculata is a local medicinal plant used in ethnomedicine for the treatment of constipation, bronchitis and asthma, treat stomach-ache and to expel intestinal worms. The aqueous decoction and the methanol extracts were subjected to anti-inflammatory activity using experimental animal model, in the presence of the positive control drugs. The roots are used in the treatment of gonorrhoea and to increase milk production in breast-feeding women. *Euphorbia geniculata* is native to Central and South America, but now widely distributed throughout the tropics and subtropics. It occurs throughout most of tropical Africa and the Indian Ocean islands, as well as in the Mediterranean Region and South Africa. *Euphorbia* comprises about 2000 species and has a worldwide distribution, with at least 750 species occurring in continental Africa and about 150 species in Madagascar and the Indian Ocean islands.

An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH), ABTS Radical Scavenging Activity, Hydrogen Peroxide Radical Scavenging Activity, Superoxide Radical Scavenging Activity, Hydroxyl Radical Scavenging Activity, Lipid Peroxidation Assay, and Nitric Oxide Radical Scavenging Activity. The present study has been centered on evaluating the *in vitro* antioxidant activity of leaves of *Euphorbia geniculata.*

MATERIALS AND METHODS

Chemicals

ABTS (2, 2'-azinobis [3-ethylbenzothiazoline-6-sulphonate]), DPPH (1, 1-diphenyl-2 picrylhydrazyl) were purchased from Sigma Aldrich Co, St Louis, USA. All other chemicals and reagents used were of analytical grade and obtained from Himedia, India.

Plant collection

The fresh leaves of *Euphorbia geniculata* was collected from the Gardens of Kumarapalayam, Namakkal District. Leaves were taken for the investigation of *in vitro* antioxidant activity. The plant material was dried in shade, coarsely powdered and was used for the extraction.

Preparation of leaves extract

The shade dried coarsely powdered leaves of *Euphorbia geniculata* (20g) was immersed in 200 ml of organic solvent (methanol and chloroform). The flask was then plugged with cotton wool and kept at room temperature for 48 hrs. After two days, the supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume. The residue obtained was stored at 4°C in air tight bottles.

Radical scavenging activity

The efficacy of methanol and chloroform extract of *Euphorbia geniculata* leaves were studied under *in vitro* conditions. The free radical scavenging activity of the leaves of *Euphorbia geniculata* against 1, 1-diphenyl-2-picrylhydrazyl (DPPH)[9], 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS)[10], hydrogen peroxide[11], super oxide[12], hydroxyl[13] and nitric oxide radical[14], and lipid peroxidation assay[15] were studied.

Statistical analysis

The biochemical parameters studied were subjected to statistical analysis using Sigma Stat statistical package (Version3.1). The experimental results are the average of triplicate experiments and

represented as mean ± standard deviation.

RESULTS AND DISCUSSION

Several concentrations ranging from100-400µg/ml of the methanol and chloroform extract of the leaves of *Euphorbia geniculata* were tested for their antioxidant activity in different *in vitro* models.

DPPH Radical Scavenging Activity

The activity of DPPH radical scavenging of methanol and chloroform extract of *Euphorbia geniculata* was presented in Figure 1. Now-a-days, antioxidants that exhibit DPPH radical scavenging activity are increasingly receiving attention[16]. The IC_{50} values for DPPH radical scavenging activity of methanol and chloroform extract of *Euphorbia geniculata* leaves were 125µg/ml and 205µg/ml respectively. DPPH radical scavenging activities of the extracts depended not only on plant type but also upon the extraction solvent. In general, DPPH scavenging activities increased with increasing phenolic content. DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. DPPH is a relatively stable nitrogen centered free radical that easily accepts an electron by reacting with suitable reducing agents. As a result, the electrons become paired off and the DPPH solution losses its violet color depending on the number of electrons taken up. The decrease in the absorbance of DPPH radical after the addition of plant extract was measured at 520 nm appearing as a deep violet color[17].

ABTS Radical Scavenging Activity

Figure 2 shows the ABTS radical scavenging activity of methanol and chloroform extract of *Euphorbia geniculata.* The IC_{50} values for ABTS scavenging activity for methanol and chloroform extract of *Euphorbia geniculata* were 175µg/ml and 250µg/ml respectively. The ABTS radical assay is suitable for both organic and aqueous solvent systems[18]. The ability of this plant extract to scavenge DPPH could also reflect its ability to inhibit the formation of $ABTS^{+}$. The scavenging activity of $ABTS^{+}$ radical by the plant extract was found to be appreciable; this implies that the plant extract may be useful for treating radical related pathological damage especially at higher concentration[19]. It is known that ABTS is an excellent substrate for peroxidases frequently used to study antioxidant properties of natural compounds[20].

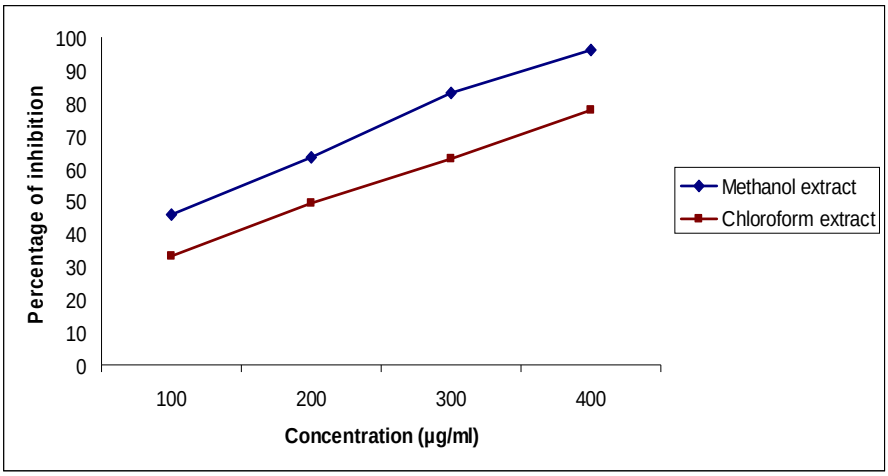

Fig. 1: DPPH radical scavenging activity of methanol and chloroform extract of *Euphorbia geniculata* Hydrogen Peroxide Radical Scavenging Activity

Figure 3 shows the H_2O_2 radical scavenging activity of methanol and chloroform extract of *Euphorbia geniculata.* Hydrogen peroxide is an important reactive oxygen species because of its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell[21]. The IC_{50} values for H_2O_2 radical scavenging activity of methanol and chloroform extract of *Euphorbia geniculata* were 165µg/ml and 240µg/ml respectively. The extract was capable of scavenging hydrogen peroxide in a concentration dependent manner. Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H2O2, thus neutralizing it to water[22,23].

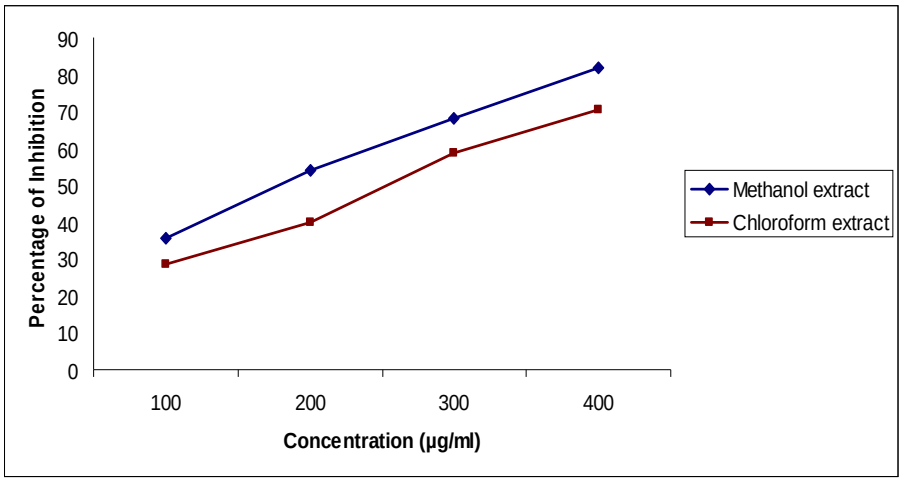

Fig. 2: ABTS radical scavenging activity of methanol and chloroform extract of *Euphorbia geniculata*

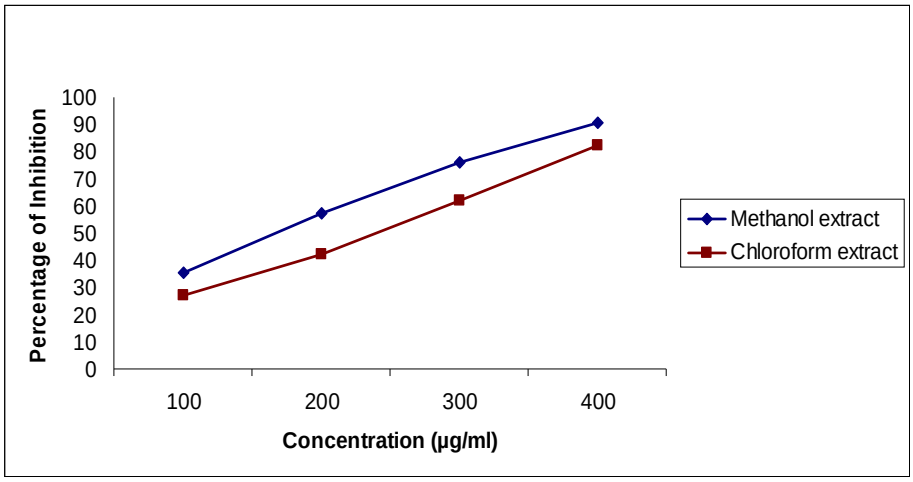

Fig. 3: H_2O_2 radical scavenging activity of methanol and chloroform extract of *Euphorbia geniculata*

Superoxide Radical Scavenging Activity

The superoxide radical scavenging activity of methanol and chloroform extract was presented Figure 4. The IC_{50} values for superoxide radical scavenging activity of methanol and chloroform extract of *Euphorbia geniculata* were 185µg/ml and 270µg/ml respectively.

Superoxide dismutase catalyzes the dismutation of highly reactive superoxide anion to oxygen and hydrogen peroxide[24]. Superoxide anion is the first reduction product of oxygen [25]. This is measured in terms of inhibition of generation of O_2.

Superoxide anion is one of the most representative free radicals. In cellular oxidation reactions, superoxide radicals have their initial effects magnified because they produce other kinds of cell-damaging free cells and oxidizing agents. In biochemical systems, superoxide radical can be converted into hydrogen peroxide by the action of superoxide dismutase and the H_2O_2 can subsequently generate extremely reactive hydroxyl radicals in the presence of certain transition metal ion [26].

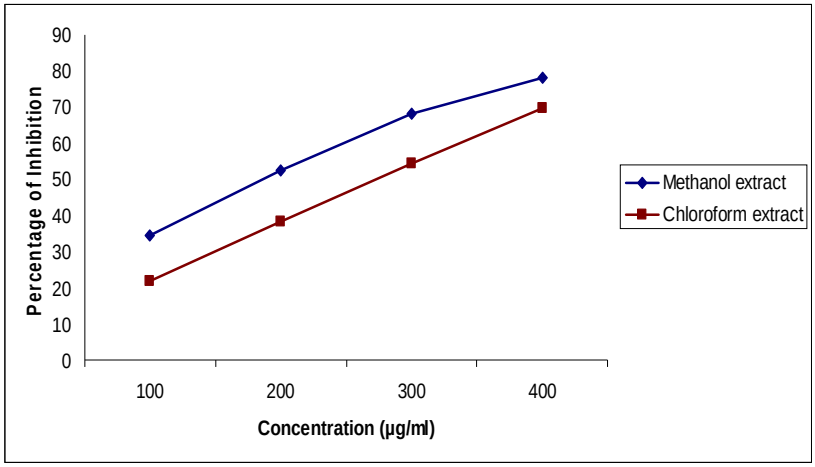

Fig. 4: Superoxide radical scavenging activity of methanol and chloroform extract of *Euphorbia geniculata*

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity of methanol and chloroform extract was presented in Figure 5. The IC_{50} values of hydroxyl radical scavenging activity of methanol and chloroform extract of *Euphorbia geniculata* were 230µg/ml and 285µg/ml respectively. Hydroxyl radicals can attack DNA molecules to cause strand scission. Hydroxyl radicals are the most reactive and predominant radicals generated endogenously during aerobic metabolism among the reactive oxygen species, which could be formed from superoxide anion and hydrogen peroxide, in the presence of metal ions, such as copper or iron and cause the aging of human body and some diseases[27]. The hydroxyl radical in cells can easily cross cell membranes at specific sites and react with most biomolecules, which further causes tissue damages and cell death. It is thus important to remove the hydroxyl radical for the protection of living systems[28]. The hydroxyl radical scavenging assay is often used for evaluating the total antioxidant power of single compounds and complex mixtures of plants.

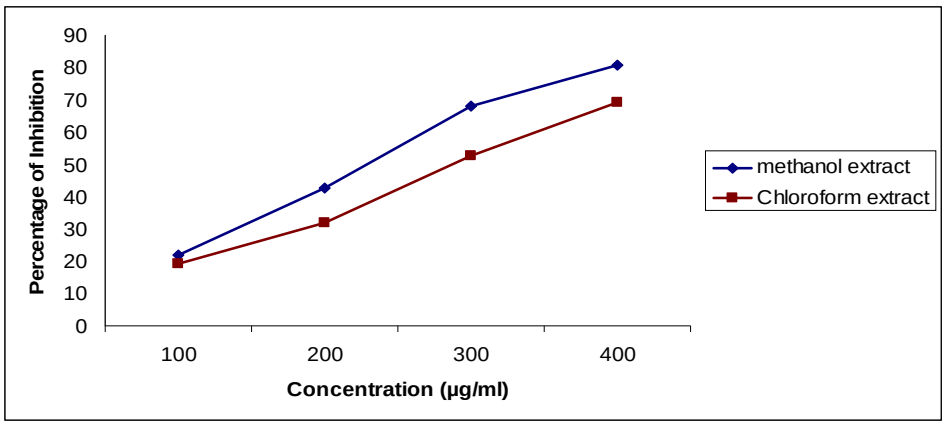

Fig. 5: Hydroxyl radical scavenging activity of methanol and chloroform extract of *Euphorbia geniculata*

Lipid Peroxidation Assay

Anti-lipid peroxidation free radicals scavenging activity was depicted in Figure 6. The IC_{50} values for anti-lipid peroxidation free radicals scavenging activity for methanol and chloroform extract of *Euphorbia geniculata* were 205µg/ml and 280µg/ml respectively. Phenolic compounds are associated with antioxidant activity or free radical scavenging and play an important role in lipid peroxidation[29]. The peroxidation of membrane lipids initiated by oxygen radicals may lead to cell injury. Initiation of lipid peroxidation by ferrous sulphate takes place either through ferryl-perferryl complex[30] or through-OH radicals by Fenton reaction[31] thereby initiating a cascade of oxidative reactions.

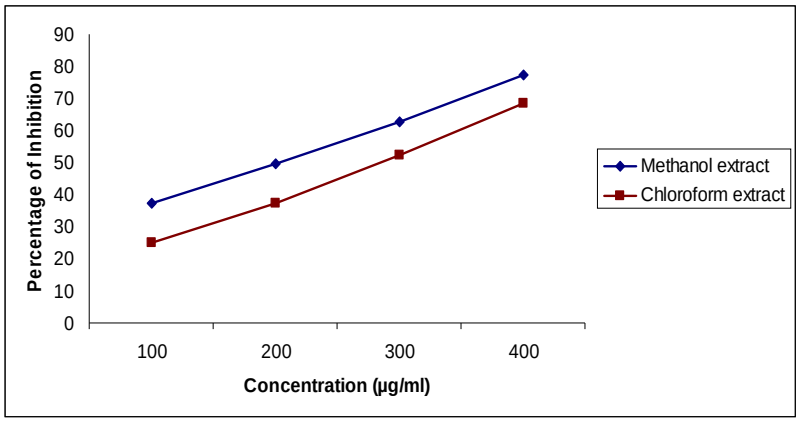

Fig. 6: Lipid peroxidation assay of ethanol and chloroform extract of *Euphorbia geniculata*

Nitric Oxide Radical Scavenging Activity

Figure 7 depicts the percentage of inhibition of nitric oxide by methanol and chloroform extract of *Euphorbia geniculata*. The IC_{50} values for nitric oxide radical scavenging activity for methanol and chloroform extract of *Euphorbia geniculata* were 255µg/ml and 280µg/ml respectively. Nitric oxide (NO) is a free radicals product in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases[32]. NO is a reactive free radical produced by phagocytes and endothelial cells, to yield more reactive species such as peroxynitrite which can be decomposed to form OH radical. In addition to reactive oxygen species, NO is also implicated in inflammation, cancer and other pathological conditions[22].

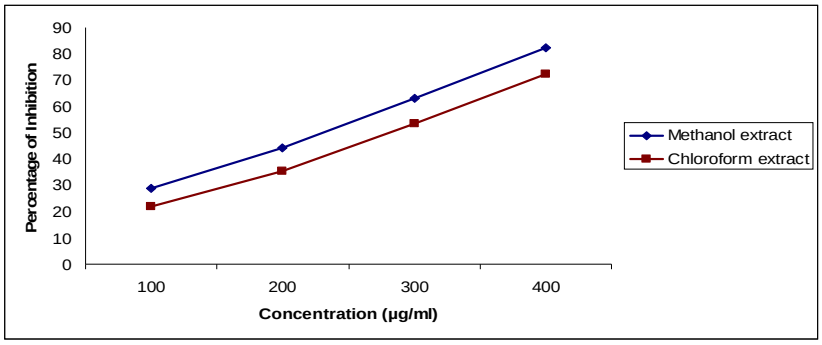

Fig. 7: Nitric oxide radical scavenging activity of methanol and chloroform extract of *Euphorbia geniculata*

CONCLUSION

In the present study, the antioxidant activity of *Euphorbia geniculata* was assessed under *in vitro* conditions. Results of the present study suggest that methanol extract of *Euphorbia geniculata* possess potent antioxidant activity and/or free radical scavenging activity than chloroform extract. More detailed studies on chemical composition of the plant extracts, as well as other *in vivo* assays are essential to characterize them as biological antioxidants which are beyond the scope of this study. The findings of this study support the view that some medicinal plants are promising sources of potential antioxidant and may be efficient as preventive agents in some diseases.

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