

RESEARCH ARTICLE

Evaluation of photochemical and free radical scavenging properties of *Euphorbia antiquorum* L. extracts

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ABSTRACT

The present study deals with evaluation of free radical scavenging ability and preliminary phytochemical screening of *Euphorbia antiquorum*. Various extracts were prepared and tested for preliminary phytochemical screening for detection of presence of various classes of chemical principles viz., flavonoids, tannins and phenolic compounds. *Euphorbia antiquorum* contains a variety of phytochemical compounds, among the extracts used ethanol and acetone extracts exhibited significant free radical scavenging activity and presence of high level of phenolic compounds which can effectively prevent free radical mediated cell damage by free radicals scavenging activity and thus can be used as a potent source of natural antioxidant compounds.

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INTRODUCTION

Free radicals are formed constantly by the body's normal use of oxygen, such as for respiration and some cell mediated immune functions. Free radicals can damage cell membranes and other vital cell components, such as genetic material in the cell nucleus and inactivate enzymes. Damage to body cells and molecules by oxygen containing free radicals have been implicated in a wide variety of diseases. Free radicals generated are detoxified by the antioxidants present in the body.

Antioxidants are quenchers of free radicals that are responsible for inducing degenerative diseases such as cancer, diabetes and cardio-vascular diseases etc. In treatment of these diseases, antioxidant therapy has gained an immense importance. There are some synthetic antioxidant compounds suspected to have some toxic effects such as carcinogenicity (Frankel, 1995). Since ancient times, the medicinal plants have been extensively used in the Indian traditional (Ayurveda) system of medicine for the treatment of different diseases of humans. Medicinal properties of plants have also been investigated in the recent scientific developments throughout

the world, due to their potent pharmacological activities, low toxicity and economic viability, when compared with synthetic drugs (Vadlapudi and Naidu, 2010). Therefore, research for the determination, development and utilization of more effective antioxidants of natural origin is desired.

Phytochemicals have shown to have free radical scavenging properties (Anonymous, 1948; Birdi *et al.*, 2006) but generally there is still a demand to find more information concerning the antioxidant potential of plant species. Plants belonging to Euphorbiaceae are reported to have antioxidant principles (Trease and Evans, 2001; Lanhers *et al.*, 1991). *Euphorbia antiquorum* L. is one among them which is a large shrub or small tree. Since the pharmacological profile of *Euphorbia antiquorum* L. is not adequately evaluated hence the present study was aimed to assess the antioxidant potential together with their phytochemical screening.

MATERIAL AND METHODS

The study plant was collected and identified with the help of available literature and comparing with the already identified plant specimens. The aerial part the study plant

was shade dried, powdered, different solvent extracts were prepared and used for screening phytoconstituents quantitatively and evaluating free radical scavenging activity by following standard methodology :

Quantitative phytochemical analysis :

Determination of total phenolics and tannins, total flavonoid contents were quantitatively analysed by the method of Siddhuraju and Becker (2003) and Zhishen *et al.* (1999).

Antioxidant studies :

*Free radical scavenging activity on DPPH** (Blios, 1958) :

A methanol solution of the sample extracts at various concentrations (60-300µg) was added to 5 ml of 0.1 mM methanolic solution of DPPH* and allowed to stand for 20 min at 27°C. The absorbance of the sample was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated by using the formula :

$$\text{DPPH radical scavenging activity percent} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Superoxide radical scavenging activity (Beauchamp and Fridovich, 1971) :

Each 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20mg riboflavin, 12 mM EDTA, 0.1 mg NBT and various concentrations (60-300 µg) of sample extracts. Reaction was started by illuminating the reaction mixture with sample extract for 90 seconds. Immediately after illumination, the absorbance was measured at 590nm. The percentage inhibition of superoxide anion generation was calculated as :

$$\text{Percent inhibition} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

The analysis was performed in triplicate. The sample concentration providing 50 per cent inhibition (IC_{50}) under the assay condition was calculated from the standard graph of inhibition percentage against sample concentration.

RESULTS AND DISCUSSION

The results obtained from the present investigation as

well as relevant discussion have been summarized under following heads :

Phytochemical analysis :

Quantitative phytochemical analysis of selected plant extracts showed the presence of total phenolics, flavonoids and tannin content (Table 1). Among various extracts used, ethanol extracts exhibited maximum values. It has been known that antioxidant activity of plants might be due to their phenolic compounds (Hanto *et al.*, 1988; Zielinski and Kozłowska, 2008). Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging activity (Mazza, 1998). The presence of polyphenolic compound in the selected plant prompted to study the free radical scavenging activity. The medicinal value of plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body (Akinmoladun *et al.*, 2007). Preliminary phytochemical investigation of Wagner *et al.* (1986) and Bandara *et al.* (1989) revealed the presence of triterpenes and flavonoids in *Euphorbia antiquorum*. They were known to show medicinal activity as well as exhibiting physiological activity (Sofowara, 1993).

Free-radical scavenging activity :

Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolics, lignins, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalins and other metabolites, which are rich in antioxidant activity (Zheng and Wang, 2001; Cai *et al.*, 2003). In the present study various solvent extracts of *Euphorbia antiquorum* showed high free radical scavenging properties.

DPPH* :

Fig.1 shows the IC_{50} value of the study plant extracts (acetone, ethanol and water) on DPPH* radical scavenging activity. The sample was compared with the standards quercetin and tannic acid. The highest DPPH* radical scavenging potential as indicated by the lowest IC_{50} value was recorded for the acetone extract followed by ethanol extract. Acetone extract of the study plant showed better percentage of inhibition of DPPH* radicals which was more or less equal to the standards. Contradictory results were recorded

Table 1 : Estimation of total phenolics, tannin and total flavonoid content of successive solvent extracts of *Euphorbia antiquorum*

Sr. No.	Extraction medium	Total phenolics (mg TAE/g extract) [#]	Tannin (mgTAE/g extract) [#]	Total flavonoid (mgRE /g extract) [#]
1.	Petroleum ether	35.65 ± 0.95	6.86 ± 0.83	0.49 ± 0.02
2.	Chloroform	26.76 ± 0.73	8.65 ± 0.57	0.84 ± 0.08
3.	Acetone	90.51 ± 0.88	11.16 ± 0.13	1.32 ± 0.05
4.	Ethanol	100.61 ± 0.83	13.30 ± 0.19	1.97 ± 0.13
5.	Water	49.02 ± 0.83	10.42 ± 0.19	1.07 ± 0.06

Values are means of three independent analyses of the extract ± Standard deviation (n=3).
TAE-Tannic acid equivalent RE-Rutin equivalent

by Siriwardhana *et al.* (2003) and Adedapo *et al.* (2009), they reported that water and methanol extracts of *Hizikia fusiformis* and *Celtis africana* showed higher DPPH scavenging activities.

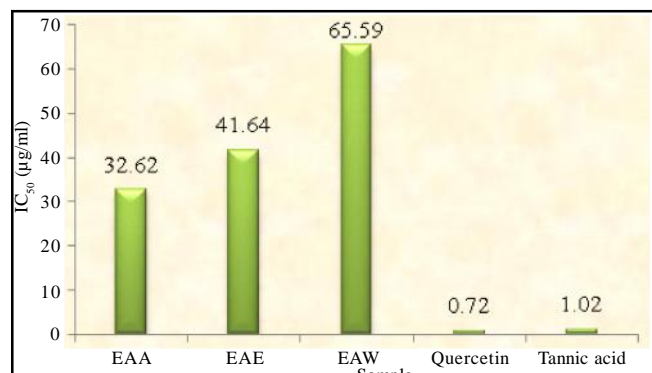
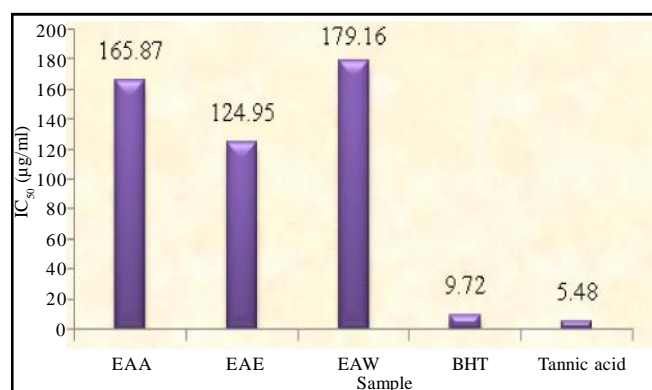


Fig. 1 : DPPH[•] radical scavenging activity of different solvent extracts of *Euphorbia antiquorum*

Superoxide radical scavenging activity :

In the present study it is noteworthy to mention that the ethanolic extract of *Euphorbia antiquorum* (Fig. 2) recorded the highest superoxide anion scavenging activity than other extracts. According to Kumaran and Joel Karunakaran (2007), the methanolic extract of *Phyllanthus debilis* possessed highest superoxide anion scavenging activity. This is in agreement with the present investigation. Nardi *et al.* (2003) reported that the crude extracts of *Croton celtidifolius* showed higher superoxide radical scavenging activity and Jyothi *et al.* (2007) reported that aqueous extract of the aerial part of *Euphorbia antiquorum* exerted significant superoxide anion radical scavenging activity. These results were contradictory



Values are means of triplicate determinations ± Standard Deviation
 EAA - Acetone extract of *Euphorbia antiquorum*
 EAE - Ethanolic extract of *Euphorbia antiquorum*
 EAW - Water extract of *Euphorbia antiquorum*
 BHT - Butylated Hydroxytoluene

Fig. 2 : Superoxide radical scavenging activity of different solvent extracts of *Euphorbia antiquorum*

with the present work. This may likely due to differences in methodology of sample extraction that can result in a wide variation in the antioxidant activity of the extract (Nuutila *et al.*, 2003).

Conclusion :

Ethanol and acetone extracts of *Euphorbia antiquorum* contained relatively larger amount of phytoconstituents and exhibited higher free radical scavenging potentials compared to other extracts. These secondary metabolites have been found useful in both preventive and curative medicine. The data obtained in the present work confirm the relatedness of the investigated study plant that revealed its potential in the therapeutic and protective effects in many diseases.

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