Phytochemical screening and Antioxidant activity of *Euphorbia caducifolia* extracts

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Abstract

Purpose: This study was conducted to screen the phytochemical compounds present in the phylloclade of *Euphorbia caducifolia* Haines and to measure the ability of free radical scavenging activity as an antioxidant. **Methods:** The collected plant phylloclades were properly dried, powdered and extracted with hexane, methanol, and water. Extracts were tested for phytochemicals, total phenolic and flavonoid content, and antioxidant activities. **Results:** Carbohydrates, phenols, flavonoids, tannins, glycosides were present in water extract and terpenoids along with phenols and flavonoids in methanol. Hexane contains a maximum of terpenoids and reducing sugars. Water extract showed maximum DPPH˙ free radical (86±1.5%) and nitric oxide scavenging activities (39.23±4.7%) followed by methanol and hexane due to the presence of phenolic (68.740 ± 0.025 mg of GAE/g) and flavonoid contents (50.04±0.125 mg of RE/g). **Conclusion:** This study suggests that *Euphorbia caducifolia* is a promising plant for the therapeutic purposes due to its antioxidant activity of water extract and high contents of terpenoids in methanol and water extracts. A further study of isolation and characterization of active compounds from the plant is necessary to treat various diseases and for multiple applications.

Keywords: *Euphorbia caducifolia*, Free radical scavenging activity, Phylloclade, Phenolic content, Flavonoids.

Introduction

Plants are a rich source of useful drugs since ancient times. In India, plants have been used for many therapeutic applications by our traditional system of medicines such as Ayurveda, Unani, Homeopathy, and Siddha. Traditional medicine system gave birth to many active compounds in early years of natural chemistry, but reports of natural products from different sources has been decreased rapidly in last decade and looking for new active compounds [1][2]. According to National Medicinal Plant Board (NMPB), a maximum number of plants using in the folk medicine compared to other documented medicine systems [3]. Now it needs to explore new natural compounds from potential folk medicinal plants and ethnomedicinal plants for better therapeutic applications.

All living organisms contain an antioxidant defensive mechanism to counter the free radicals, reactive oxygen species (ROS), reactive nitrogen species (RNS) and other oxidants produced as byproducts of the metabolism. Most of the diseases caused by overproduction and reactive mechanism of free radicals [4][5]. Many medicinal plants, spices, aromatic plants using as food supplements and medicines are natural antioxidants [6]. It has been an upsurge of interest for potential antioxidants from plants sources.

Euphorbia caducifolia Haines (leafless milk hedge), a folk medicinal plant belongs to Euphorbiaceae (Castor family). It is a latex-producing plant and found in rocky areas of the tropical region [7]. The plant latex is used for wound healing, leucoderma, and skin eruptions [8]. Latex and root of the plant used to treat cancer [9]. Root also used for snakebite in Maharashtra [10]. Leaf extracts of the plant were reported for its antimicrobial activity [11]. Apart, there are not many reports on phylloclade of the plant which are a rich source of biofuel and other compounds [12]. Therefore, the present study was conducted to reveal the phytochemical compounds exist in the phylloclade and to explore the ability of free radical scavenging activity as an antioxidant.

Materials and Methods

Collection of Plant material

The aboveground parts of *Euphorbia caducifolia* plant were collected from Bhongiri rocky areas, Yadadri district of Telangana (Fig. 1) and shade dried until the material obtains a constant weight.

Preparation of Extracts

The dried plant material was made into powder and used for extraction. The powder was extracted with hexane, methanol, and water in successive order. The extracts were concentrated at temperature 40°C under vacuum and used for further analysis.

Phytochemical screening
Extracts were analyzed for alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, quinones, tannins, saponins, and terpenoids [13].

Estimation of Total Phenolic Content (TPC)
The TPC of the extracts was determined using the Folin-Ciocalteu colorimetric method. 1 ml of extract was mixed with 1 ml of 1 N Folins reagents and well shaken. After 5 min, 1 ml of 10% sodium carbonate was added and incubated for 1 h at room temperature. The absorbance was taken after incubation at 760 nm against blank. Total phenolic content was given as Gallic acid equivalents (GAE) [14].

Estimation of Flavonoids
0.1 ml of extract was added to 0.3 ml 5% sodium nitrite and mixed with 3 ml of 1% aluminum chloride. After 5 min incubation, 2 ml of 1 M NaOH was added and made up the volume to 10 ml with water. The solution was mixed well and measured absorbance at 510 nm against blank. The flavonoid content was calculated using rutin as standard [14].

Antioxidant Activity

DPPH Scavenging Assay
DPPH (2,2-diphenyl 1-picrylhydrazyl) free radical used to determine the scavenging activity of extracts [15]. DPPH (1 ml, 0.1mM) added to various concentrations of extracts and BHT (3 ml) and 0.5 h incubated in the dark and measured absorbance at 517 nm against the blank (n=3). The activity was calculated by the following equation [16]:

Percentage inhibition = [(Abs control – Abs sample)/Abs control] × 100

Nitric oxide radical scavenging Activity
Nitric oxide (NO) produced by Sodium Nitroprusside at pH 7.2-7.4 was determined by Griess reagent (Griess diazotization reaction). The reaction mixture (3 ml) was incubated at 25°C for 2 hr containing Sodium Nitroprusside (10 mM) and extracts with different concentrations. An Aliquot (0.5 ml) was added to Griess reagent (0.5 ml) and measured at 546nm (n=3). The activity was calculated by comparing control and test sample. Ascorbic acid used as reference compound [17].

Results and Discussion

Phytochemical analysis
Phytochemical studies of extracts showed the presence of various classes of metabolites. *E. caducifolia* showed the high quantity of terpenoids in methanol and hexane extracts as *Euphorbia* species are predominant with cyclic and acyclic terpenoids [18]. Test for the presence of alkaloids shown no significant colouration. Water extract was rich in carbohydrates, glycosides, tannins, phenols, flavonoids and saponins. Further experiments revealed that the presence of acidic polysaccharides and saponin glycosides. Methanol extract contained reducing sugars, glycosides and some phenolic compounds whereas in hexane extract showed positive for reducing sugars and glycosides along with terpenoids (Table 1).

Estimation of total phenolic and flavonoid content
Phenolic compounds such as flavonoids and tannins are excellent free radical scavengers and protect the plant from different types of oxidative damages [19]. Water extract showed the high phenolic and flavonoid contents whereas no significant amounts observed in hexane extract. Methanol extract showed good amounts of flavonoids compared to phenolics (Table 2). Antioxidant activity of the extracts might be due to the presence of phenols and flavonoids present [20][21].

Antioxidant activity
Free radicals are the most reactive molecules and damage the cells. Aerobic organisms protect themselves from free radical by the defensive antioxidant mechanism, and it is necessary to provide external antioxidants in failure defensive system [22]. The extracts were analyzed for their antioxidant capacity to use as a free radical scavenger. Most of the members of the castor family are a good source of natural antioxidants such as *Euphorbia hirta* [14], *E. heterophylla* [23]. *E. caducifolia* extracts also exhibited antioxidant activity. The potentiality of the extract to scavenge the free radical produced was considered for antioxidant activity (Fig. 2). DPPH and NO are the two radicals used for the experiments. All extracts were showed activity on concentration dependency. Water extract exhibited more scavenging activity towards DPPH (86±1.5%) as well NO (39.23±4.7%) to hexane extract. More free scavenging activity of water extract might be due to the presence of the high phenolic and

flavonoid contents. Methanol extract also exhibited similar (DPPH-77.81±1.3% and NOSA-36.03±2.71%) to water but dropped activity frequency at high concentration of the extract. Higher antioxidant activity was observed at higher concentrations (Fig. 3). Extracts showed less ability to scavenge the NO free radical as compared to DPPH.

Conclusion

Euphorbia caducifolia is a promising plant for the various therapeutic applications with rich contents of terpenoids, polysaccharides, tannins, flavonoids and phenolic compounds. The presence of significant amount of total phenolics and flavonoids contents in water extract might be responsible for strong antioxidant activity as compared with methanol and hexane extract. Hexane and methanol extract can be used for the terpenoid sources and water extract for phenolics and flavonoids. High contents of terpenoids in methanol and hexane extracts are useful in various applications. A further study of isolation and characterization of bioactive molecules from the plant is required for therapeutic and multiple applications.

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Table 1: Phytochemical Screening of *E. caducifolia* extracts

Fraction	Water	Methanol	Hexane
Alkaloids	--	--	--
Carbohydrates	++	++	++
Glycosides	++	++	++
Flavanoids	++	+	--
Phenolics	++	+	--
Tannins	++	--	--
Saponins	++	--	--
Quinones	--	++	++
Terpenoids	--	++	++

Table 2: Total Phenolic and Flavonoid contents in *E. caducifolia* plant extracts

Fraction	Phenolics	Flavonoids
	mg of GAE/g of Extract	mg of RE/g of Extract
Water	68.740 ± 0.025	50.04 ± 0.125
Methanol	00.926 ± 0.06	13.534 ± 0.754
Hexane	00.080 ± 0.014	00.004 ± 0.0002

(n=3, Mean ± SD)

Figure 1: Distribution of *Euphorbia caducifolia* in the wild.

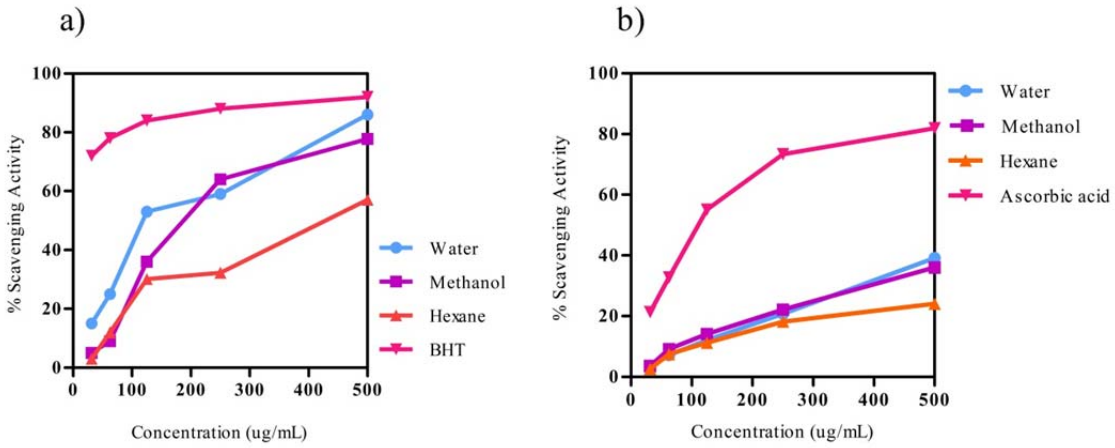

Figure 2: Antioxidant activity of *E. caducifolia* plant extracts a) DPPH Scavenging activity, b) Nitric Oxide Scavenging activity

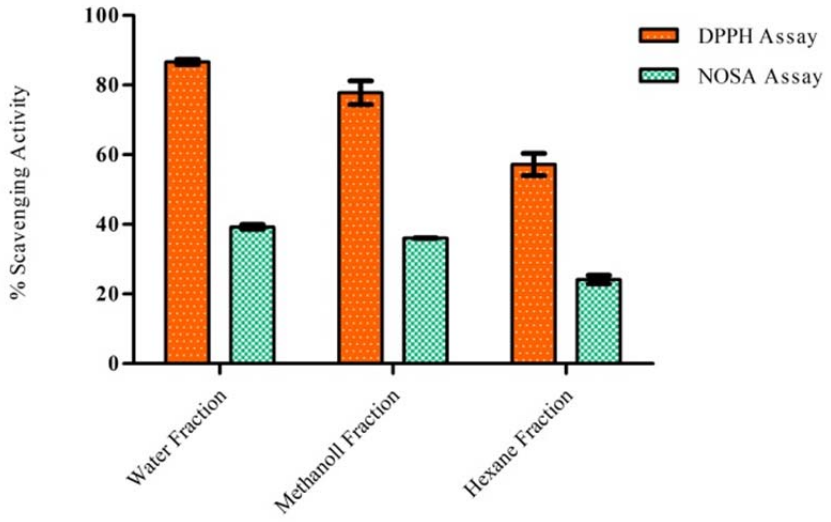

Figure 3: Antioxidant activity at maximum concentration (500 µg/ml) of *E. caducifolia* plant extracts