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A Study on Phytochemical Screening and Antioxidant Properties of Aqueous Extracts of *Euphorbia milli*

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Abstract

Different species of *Euphorbia are* used for the treatment of various ailments such as skin diseases, intestinal parasites and warts. Moreover, some species of *Euphorbia* have been traditionally used for the treatment of skin diseases, gonorrhea, migraine, intestinal parasites and as wart cures. Therefore, current study aimed for phytochemical screening and evaluation of antioxidant properties aqueous extract of stem parts of *E. milli.* Stem parts of *E* were selected for phytochemical screening and subjected to successive solvent extraction by continuous hot Soxhlet extraction with distilled water. The major phytochemicals found in aqueous extract of stem parts of *E.mini* were, steroids/phytosterols, anthocyanin and betacyanin, terpenoids, flavonoids and tannins. The IC_{50} values exhibited by aqueous extracts of stem parts of *E.milii* was 138.44 pg/ml. In conclusion, this preliminary study confirms the presence of various secondary metabolites *E. mill.* Biological activity such as antioxidant properties of aqueous extracts of stem parts of *E. milii* inferred that *E. mail* could be exploited as prospective drug agent of folk medicines.

Keywords: Phytochemical screening, *Euphorbia milii,* Antioxidants, Steroids, Flavonoids

INTRODUCTION

The family euphorbiaceae consists of 2000 species [1]. The genus euphorbia is the largest genus of medical plant. *Euphorbia milii is* commonly known as "crown of thrown" [2]. The part of plants that grow above ground that was used for medicinal purposes. The genus *E. milii* is the largest genus of medicinal plants widely distributed in tropical countries.

Different species of *Euphorbia* are used for the treatment of various ailments such as skin diseases, intestinal parasites and warts. It has been reported that *Euphorbia* possesses anti arthritis, anticancer, anticonvulsant, ant diabetic, anti-eczema, anti-inflammatory, antimicrobial, antioxidant, antispasmodic, antitumor, antitussive properties, hormonal and myelopoiesis properties [3]. Some species of *Euphorbia* have been traditionally used for the treatment of skin diseases, gonorrhea, migraine, intestinal parasites and as wart cures [4]. The genus *Euphorbia* has been studied widely for its antiproliferative [5].

Fungi of *the* genus *Aspergillus* produce a toxic substance called aflatoxin, which contaminates crops *(e.g.,* corn and *peanuts)* and causes human diseases. Aflatoxin has even *been* implicated *as* a contributing factor in *liver cancer*. *E. milli* flowers, when dried and processed as powder, inhibit the growth of *Aspergillus[2]*. Milin, an extract of *E. milii* latex, is a glycosylated serine protease (an enzyme that breaks down protein and has a sugar attached to it). Because it is more stable than most proteases, it will be useful to food processers and makers of detergents who have been using proteases in their operations [2,6].

Phytochemical studies of *E. milli* revealed the presence of flavonoids, terpenoids, and tannins. Flavanoids are yellow pigments, which occur in plant kingdom either in free state or as a glycosides or associated with tannins. These are known as anthoxanthins [7]. With this background, the present study was undertaken for phytochemical screening and evaluation of antioxidant properties of *E. mild.*

MATERIALS AND METHODS

Collection of plant material and processing

Aerial plant of E *mini* were collected from natural habitats of Chikkaballapura districts of Karnataka State. Stem parts were separated from *E. milli* plant material collected and sprayed with ethanol. Then shade dried at room temperature for 10 days. The dried stein pails were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis [2.8]

Extraction

Approximately 50g. of dried and coarsely powdered stem parts of *E milli* was subjected to successive solvent extractionby continuous hot extraction (Soxhlet) with 330 ml, of double distilled water. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The extracts wore preserved in airtight containers and stored at room temperature until further use.

Phytochemical screening

Phytochemical screening was carried out on the aqueous extracts of thorn and stem parts of *E. mifii* by using standard Procedure to detect constituents as described by Sofora 191, Trease and Evans' I0J, and Harbortief I 1J.

Alkaloids

Approximately 0.2g of aqueous extract astern parts of E, *milli* was warmed with 2% HISO, (2.0m1) for two minutes. The reaction mixture was filtered and few drops of Dragendrors reagent was added to the filtrate. Orange red precipitation showed the presence of alkaloids moiety.

Tannins

The aqueous extract of stem parts of *E. milifin* small quantity was mixed with water and heated on water bath and filtered. To the filtrate, few drops of ferric chloride ($FeC1_3$) was added. A dark green colorationindicates the presence of tannins.

Anthroquinone

Approximately 0.5g of aqueous extract of stem parts of *E. milli* was boiled with 10% HO for few minutes_ The reaction mixture was then filtered and allowed to cool. Equal volume of chloroform (CHCI3) was added to each *filtrate along* with few drops of 10% NH3 and heated. Rose-pink color formation was obtained which indicate the presence of anthraquinones.

Glycosides

About 0.6g of aqueous extract of stem parts of *E. milli* was hydrolyzed with HO and neutralized with NaOH solution and few drops of Fehling's solution A and B were added. Formation of red precipitate indicates the presence of glycosides.

Reducing sugars

The aqueous extract of stem parts of *E. milli was* shaken with distilled water and filtered. Few drops of Fehling's solution A and B were added and boiled for few minutes. Formation of an orange red precipitate confirms the presence of reducing sugar.

Saponins

About 0.2g of aqueous extract of stem parts of *E. milli* was shaken with 5 mL of distilled water and then heated to boil_Frothing (appearance of creamy miss of small bubbles) showed the presence of saponins.

Flavonoids

0.2g of aqueous extract of stem parts of *E. milli* was dissolved in diluted 10%Na0FI and few drops of 2M HCI was added. A yellow solution that turns into colorless indicate the presence of flavonoids.

Phlobatanins

About 0.5g of aqueous extract of stem parts of *E. milli* was dissolved in distilled water and filtered. The filtrate was then boiled with 2M HCl solution. Red precipitates showed the presence of phlobatannins.

Steroids

2 mL of acetic anhydride was added to 0.5g of aqueous extract of stem parts of *E. milli* and then added 2 mL of H_2SO_4. The change of color from violet to blue or green or red showed the presence of steroids.

Terpenoids

0.3g of aqueous extract *of stem* parts of *E. milli* was mixed with 2 mL of chloroform ($CHC1_3$) and 3 mL of concentrated $6MH_2SO_4$ was carefully added to form a layer. Reddish brown coloration at the interface was formed which indicate positive results for the presence of terpenoids.

Anthoeyattin and *Betacyanin*

To the 0,2g of aqueous extract of stem parts of *E. milli*, Na011 (2N) was added and heated for 5 mins, at 100°C. Formation of bluish green colour showed the presence of anthocyanin and betacyanin,

Proteins and Amino acids

To the 0.3g of aqueous extract of stem parts *of E, milli few* drops of 0.2% ninhydrin solution was added *and* heated for 5 minutes. Blue colorationindicates the presence of proteins.

Cardiac glyalskles

Aqueous extract of stem parts of *E. milli* was mixed with I ml of glacial acetic acid (CH3COOH) and 5% ferric chloride ($FeCl_3$) and then few drops of conc.H_2SO_4 was added. Greenish blue colour was observed which indicates the presence of glycosides.

Antioxidant assay

The modified literature protocol of Blois was used for antioxidant assay.[12,13] Briefly 2, 2-dipheny1-1-picrylhydrazyl (DPPH) solution (1m1...,1mM) was prepared in methanol and mixed with sample solution (3mL, containing 20-10Oug) in distilled water. The control was also *run* which contains only distilled water. The hydrogen atom or electron donation abilities of *each* extracts and standards were measured from the bleaching of the purple-colored methanol solution of 2, 2- diphenyll-picrylhydrazyl (DPPH). The absorbance was measured at 517 nm after 30 min incubation. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated by using the formula; DPPH% = (Control abs —Extract abs / Control) x 100. The IC_{50} value was determined by using linear regression equation *i.e.* Y = Mx + C; Here, Y=50, M and C values were derived from the linear graph trend line.

RESULTS AND DISCUSSION

The major phytochemicals found in aqueous extracts of stem parts of *E. milli* were, steroids/phytosterols, anthocyanin and betacyanin, terpenoids, flavonoids and tannins. The IC50 values exhibited by aqueous extracts of stem parts of *E. milli* was 187.91 and 138.44 respectively (Table 1 and 2).

Table 1: Photochemical screening of aqueous extracts of stem parts of *E. milli*

Chemical Components	Stem Part
Alkaloids	_
Anthocyanin and Betacyanin	+
Anthraquinone	-
Cardiac glycosides	+
Flavonoids	+
Glycosides	-
Phlobatanins	-
Proteins and Amino acids	+
Reducing sugar	-
Saponins	-
Steroids	+
Tannins	+
Terpenoids	+

Table 2: Antioxidant activities of aqueous extracts of stem parts of *E. milli*

S. No.	Extract	IC_{50} (pg/mL)
1	Stem part	138.44

The preliminary screening and evaluation of antioxidant activities of different parts of *E. milli* was undertaken in the present study due to the fact that they have been used in the folk therapy against various ailments and are rich sources of secondary metabolites and hydrocarbons, The present study revealed that the phytochemical constituents of aqueous extract of stem parts of *E. 'nail* include steroids/phytosterols, anthocyanin and betacyanin.

terpenoids, flavonoids and tannins. These findings are in accordance with results of Rauf at al except for steroids[2].

Aqueous extract of stem parts of E. *milli* showed antioxidant activities which may be due to the presence of secondary metabolite for example flavonoids, terpenoids, tannins, and phenolic compounds[14, 15,16]. The presence of phlobatannins in extracts suggests the diuretic property of the plant[1 7]. Flavonoids were found in aqueous extracts of *E. milli* prevent oxidative cell damage indicating antiseptic, anticancer, anti-inflammatory effects and mild hypersensitive properties[1 8]. Phenolic compounds present in aqueous extract of stem parts of E. *milli* are responsible for antioxidant activity[19].

The results obtained in our study are encouraging as this study evidenced the vide variety of secondary metabolites present in the aqueous extracts of stem parts of *E. milli* have shown considerable antioxidant properties. Therefore, current study findings depicted that stem parts of the *E. milli* could be exploited as folk medicine for the management of various ailments.

CONCLUSION

Our study findings demonstrated that aqueous extract of stem parts of *E. milii* has various secondary metabolites. Biological activity *such* as antioxidant properties of aqueous extracts of stem parts of *E. milli* inferred that *E. milii* could be exploited as prospective drug agent of folk medicines. Moreover, additional studies are recommended to carried out to elucidate the exact mechanism of action of various secondary metabolites present in *E. milli* against various diseases.

REFERENCES

1. Davis PH, Mill RR and Tan K_ Flora of Turkey and the East Aegean Islands. University Press, Edinburgh. 1988;10:513.
2. Rauf A, Khan A, Uddin N, Akram M, Arfan M, Uddin G, Qaisar M. Preliminary phytochemical screening, antimicrobial and antioxidant activities of *Euphorbia milli.* Pakistan journal of pharmaceutical sciences. 2014;27(4).

3. Milica P, Jasna B, Ivana SA, Nina M, Todorovic, Milka 3, Vlatka E. Vajs, Vele VT, IvanV, Miljana M, Ivanka D, Markovic, Nikola T, Sabera R. New anticancer characteristics of Jathrophanediterpenes from *Euphorbia dendroides.* Food Chemistry and Toxicology. 2011;49(12):3165-3173.
4. Mwine Ti, Van Damme P. Why do Euphorbiaceae tick as medicinal plants: a review of Euphorbiaceae family and its medicinal features. Journal of Medicnal plant Research. 2011;5(5): 652-662.
5. Chaabi M, Freund-Michel V, Frossard N, Randriantsoa A, Andriantsitohaina R, Lobstein A. Anti-proliferative effect of *Euphorbia stenoclada* in human airway smooth muscle cells in culture. Journal of Ethnopharmacol. 2007;109(1):134-139.
6. Kamurthy H, Dontha S, Rajani K: Phytochemical Screening on *Euphorbia milii* Red Flowers-Isolation of Terpenoids, Flavone and Phenols: American Journal of Ethnomedicine.2015;6:322-332.
7. Devanaboyina N, Bhavani B. Antimicrobial studies on flowers of *Euphorbia Milli:* Scholar Research Library. 2015; 3:196-204.
8. Kamurthy H, Dontha S, Rajani K: Phytochemical Screening on *Euphorbia milli* Red Flowers-Isolation of Terpenoids, Flavone and Phenols: American Journal of Ethnomedicine.2015,6:322-332.
9. Sofora A. Medicinal plants and Traditional Medicine in Afric. John Wiley and son Ltd., 1993;150-153.
10. Trease GE, Evans WC. Pharmaccology, 1 lthEdtm. Brailliar Tiridel and Macmillian Publishers, London. 1989.
11. Herbome 7B. Phytochemical Methods 3 Edn. D.E. and Hall Ltd., London. 1973;135-203.
12. Blois MS. Antioxidant determinations by the use of a stable free radical. nature. 1958;181(4617):1199-200.
13. Uddin G, Rauf A, Arfan M, All NI, Qaisar NI, Saadiq M, Atif M. Preliminary phytochemical screening and antioxidant activity of *Bergenia caliata.* Middle-East J. Sci. Res. 2012;11(8);1140-1142.

14. Uddin *G*, Rauf A, Qaisar M, Latif A, All M. Preliminary phytochemical screening and antimicrobial activity of *Hedera helix*. Middle-East J Sci Res. 2011;8(1):198-202.
15. Uddin G, Rauf A, Reiman TU, Qaisar M. Phytochemical screening of *Pistacia chinensis* var, integerrima. Middle-East 3 Sci. Res. 2011;7(5)·.707-11.
16. Rauf A, Muhammad N, Qaisar M, Uddin G, Hussain 1. Preliminary antinociceptive studies of methanol extract of *Euphorhia milli*. Middle-East I Med Plants Res. 2012;1(3)168-70.
17. Awoyinka OA, Balogun 10, Ogunnowo AA. Phytochemical screening and *in-vitro* bioactivity of *Cnidoscolus aconirifolists* (Euphorbiaceae). Journal of Medicinal Plants Research. 2007;1(3):63-5.
18. Okwu DE. Phytochemicals, vitamins and mineral contents of two Nigerian medicinal plants. Int. J. Mol. Med, Adv. Sci. 2005;1(4):375-81.
19. Pima PG. Flavonoids as antioxidants. J. Nat, Prod. 2000; 63:1035.