ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 04, Iss 05, August 2015

QUALITATIVE PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF EUPHORBIA MILII EXTRACT

Mamatha N[1], Keshamma E[2*]

[1]Associate Professor, Department of Botany, K. G. F. First Grade College, Oorgaum, K. G. F., Karnataka, India

[2]Assistant Professor, Department of Biochemistry, Maharani's Science College for Women, Palace Road, Bengaluru, Karnataka, India.

***Corresponding Author**

Dr. Keshamma E

Assistant Professor, Department of Biochemistry, Maharani's Science College for Women, Palace Road, Bengaluru, Karnataka, India.

Email: keshamma.blr76@gmail.com

ABSTRACT

Numerous Euphorbia species are used to treat a range of conditions, including warts, intestinal parasites, and skin disorders. Additionally, some species of Euphorbia have long been used as remedies for warts, gonorrhea, migraines, intestinal parasites, and skin conditions. Hence, the present study was conducted with the main purpose of phytochemical screening and evaluation of antioxidant properties aqueous (aq.) extract of stem parts of E. milii. Stem parts of E. milii were selected for phytochemical screening and subjected to successive solvent extraction by continuous hot Soxhlet extraction with double distilled water. Results revealed that the major phytochemicals found in aq. stem extract of E. milii were found to be steroids/phytosterols, anthocyanin and betacyanin, terpenoids, flavonoids and tannins. The IC_{50} values exhibited by aq. stem extract of E. milii was found to be 124.53 µg/mL. In conclusion, this preliminary study showed that the stem parts of E. milii have a variety of secondary metabolites in their aqueous extract. Based on biological activity like antioxidant properties of aqueous extract of E. milii stem parts, it is possible to use E. milii as a potential drug agent for traditional medicines. Furthermore, it is advised that more research be done to clarify the precise mode of action of the different secondary metabolites found in E. milii against different illnesses.

Keywords: Euphorbia milii, Secondary metabolites, Phytochemical screening Antioxidants, Flavonoids

INTRODUCTION

There are two thousand species in the family Euphorbiaceae.[1] The largest genus of medicinal plants is Euphorbia. "Crown of thrown" is the common name for Euphorbia milii.[2] the portion of plants used for medicine that grows above ground. The largest genus of therapeutic plants that is extensively found in tropical nations is E. milii. 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 antiarthritis, anticancer, anticonvulsant, antidiabetic, 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. milii 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. milii revealed the presence of flavonoids, terpenoids, and tannins. Flavonoids 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 scenario, in the current we aimed for the phytochemical screening and evaluation of antioxidant activities of E. milii.

MATERIALS AND METHODS

Collection Fenugreek Leaves

Aerial plant of E. milli were collected from natural habitats of Chikkaballapura districts of Karnataka State. Stem parts were separated from E. milii plant material collected and sprayed with ethanol. Then shade dried at room temperature for 10 days. The dried stem parts were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.[2,8]

Extraction

Approximately 50 g of dried and coarsely powdered stem parts of E. milii was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 550 mL of double distilled water. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The extracts were preserved in airtight containers and stored at room temperature until further use.

Phytochemical Screening

Phytochemical screening was carried out on the stem parts of E. milii by using standard procedures to detect phytoconstituents as described by Sofora,[7] Trease and Evans[8] and Herborne.[9]

Test for Alkaloids

Approximately 0.2g of aqueous (aq.) extract of stem parts of E. milii was warmed with 2% H_2SO_4 (2.0ml) for two minutes. The reaction mixture was filtered and few drops of Dragendrof's reagent was added to the filtrate. Orange red precipitation showed the presence of alkaloids moiety.

Test for Tannins

The aqueous extract of stem parts of E. milii in small quantity was mixed with water and heated on water bath and filtered. To the filtrate, few drops of ferric chloride ($FeCl_3$) was added. A dark green coloration indicates the presence of tannins.

Test for Anthraquinone

Approximately 0.5g of aqueous extract of stem parts of E. milii was boiled with 10% HCl for few minutes. The reaction mixture was then filtered and allowed to cool. Equal volume of

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 04, Iss 05, August 2015

chloroform ($CHCl_3$) was added to each filtrate along with few drops of 10% NH_3 and heated. Rose-pink color formation was obtained which indicate the presence of anthraquinones.

Test for Glycosides

About 0.6g of aqueous extract of stem parts of E. milii was hydrolyzed with HCl 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.

Test for Reducing sugars

The aqueous extract of stem parts of E. milii 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.

Test for Saponins

About 0.2g of aqueous extract of stem parts of E. milii 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.

Test for Flavonoids

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

Test for Phlobatanins

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

Test for Steroids

2 mL of acetic anhydride was added to 0.5g of aqueous extract of stem parts of E. milii 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.

Test for Terpenoids

0.3g of aqueous extract of stem parts of E. milii was mixed with 2 mL of chloroform ($CHCl_3$) and 3 mL of concentrated 6M H_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.

Test for Anthocyanin and Betacyanin

To the 0.2g of aqueous extract of stem parts of E. milii, NaOH (2N) was added and heated for 5 mins. at 100°C. Formation of bluish green colour showed the presence of anthocyanin and betacyanin.

Test for Proteins and Amino acids

To the 0.3g of aqueous extract of stem parts of E. milii few drops of 0.2% ninhydrin solution was added and heated for 5 minutes. Blue coloration indicates the presence of proteins.

Test for Cardiac glycosides

Aqueous extract of stem parts of E. milii was mixed with 1mL of glacial acetic acid (CH_3COOH) 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-

diphenyl-1-picrylhydrazyl (DPPH) solution (1mL;1mM) was prepared in methanol and mixed with sample solution (3mL, containing 20-100ug) 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-diphenyl1-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) × 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 aq. extracts of stem parts of E. milii were, steroids/phytosterols, anthocyanin and betacyanin, terpenoids, flavonoids and tannins. (Table 1).

Table 1. Qualitative phytochemical screening of aq. stem extract of E. milii

Phytochemical Components	Aq. Stem Extract of E. milii
Alkaloids	-
Anthocyanin and Betacyanin	+
Anthraquinone	-
Cardiac glycosides	+
Flavonoids	+
Glycosides	-
Phlobatanins	-
Proteins and Amino acids	+
Reducing sugar	-
Saponins	-
Steroids	+
Tannins	+
Terpenoids	+

+, Presence; -, Absence

The IC_{50} values exhibited by aq. stem extract of E. milii was found to be 124.53 µg/mL (Table 2)

Table 2. Antioxidant activities of aq. stem extract of E. milii

S. No.	Extract	IC_{50} (µg/mL)
1	Aq. Stem Extract of E. milii	124.53

Because different parts of E. milii are rich in hydrocarbons and secondary metabolites and have been used in folk medicine to treat a variety of illnesses, the present study screened and evaluated the antioxidant activity of these parts. The results of this study showed that the aqueous extract of E. milii stem parts contain a variety of phytochemical constituents, including tannins, terpenoids, flavonoids, anthocyanins, and betacyanins. These findings are in accordance with results of Rauf et al., except for steroids.[2]

Aqueous extract of stem parts of E. milii showed antioxidant activities which may be due to the presence of secondary metabolite for example flavonoids, terpenoids, tannins, and phenolic compounds.[14-16] The presence of phlobatannins in extracts suggests the diuretic property of the plant.[17] Flavonoids were found in aqueous extracts of E. milli prevent oxidative cell damage indicating antiseptic, anticancer, anti-inflammatory effects and mild hypersensitive properties.[18] Phenolic compounds present in aqueous extract of stem parts of E. milli are responsible for antioxidant activity.[19]

Our study results are promising because they demonstrate the wide range of secondary metabolites that are present in the aqueous extracts of E. milii stem parts and have demonstrated significant antioxidant qualities. Thus, the results of the current study suggested that E. milii stem parts might be used as traditional medicine to treat a variety of illnesses.

CONCLUSION

In conclusion, the results of our investigation showed that the stem parts of E. milii have a variety of secondary metabolites in their aqueous extract. Based on biological activity like antioxidant properties of aqueous extract of E. milii stem parts, it is possible to use E. milii as a potential drug agent for traditional medicines. Furthermore, it is advised that more research be done to clarify the precise mode of action of the different secondary metabolites found in E. milii against different illnesses.

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 milii. Pakistan journal of pharmaceutical sciences. 2014;27(4).
3. Milica P, Jasna B, Ivana SA, Nina M, Todorovic, Milka J, Vlatka E. Vajs, Vele VT, Ivan V, Miljana M, Ivanka D, Markovic, Nikola T, Sabera R. New anticancer characteristics of Jathropha diterpenes from Euphorbia dendroides. Food Chemistry and Toxicology. 2011;49(12):3165-3173.

4. Mwine TJ, Van Damme P. Why do Euphorbiaceae tick as medicinal plants: a review of Euphorbiaceae family and its medicinal features. Journal of Medicinal 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 Milii: Scholar Research Library. 2015; 3:196-204.
8. 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.
9. Sofora A. Medicinal plants and Traditional Medicine in Afric. John Wiley and son Ltd., 1993;150-153.
10. Trease GE, Evans WC. Pharmaccology, 11th Edtn. Brailliar Tiridel and Macmillian Publishers, London. 1989.
11. Herborne JB. 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, Ali M, Qaisar M, 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, Ali 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, Rehman TU, Qaisar M. Phytochemical screening of Pistacia chinensis var. integerrima. Middle-East J Sci Res. 2011;7(5):707-11.
16. Rauf A, Muhammad N, Qaisar M, Uddin G, Hussain I. Preliminary antinociceptive studies of methanol extract of Euphorbia milii. Middle-East J Med Plants Res. 2012;1(3):68-70.
17. Awoyinka OA, Balogun IO, Ogunnowo AA. Phytochemical screening and in-vitro bioactivity of Cnidoscolus aconitifolius (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. Pietta PG. Flavonoids as antioxidants. J. Nat. Prod. 2000; 63:1035.