Antioxidant activity, Total Phenolic and Flavonoid Contents and Cytotoxic activity of *Euphorbia aegyptiaca*

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**ABSTRACT**

*Euphorbia aegyptiaca* is a herbaceous plant traditionally used in Sudan for treatment of various diseases, and the study of this plant is still limited. The aim of the present study was to screen the phytochemicals and to assess the Antioxidant activity, total phenolic, flavonoid contents and cytotoxic activity of *Euphorbia aegyptiaca*. The plant material was extracted successively by Soxhlet apparatus using n-hexane, chloroform and methanol. The chemical constituents of the extracts were carried out using the standard procedures. The Folin-Ciocalteu and Aluminum chloride method was employed to calculate the total phenolic and flavonoid content, respectively. The antioxidant activity, was assessed by measuring the scavenging activity of the DPPH (2,2-Di (4-methoxyphenyl)-1-picolyl-hydrazyl) and Propyl Gallate as standard antioxidants. While cytotoxic activities were screened using brine shrimp. Phytochemical screening studies revealed that flavonoids, tannins, coumarins, saponins, sterols, terpenes, anthraquinones and alkaloids were the main phytochemicals present in extracts of *E. aegyptiaca*. The methanol extract showed the highest level of total phenolic contents (173.49±2.427 mg GAE/g) and flavonoid content (239.53±7.90 mg QE/g), and the highest antioxidant activity 89% with least IC\(_{50}\) 0.0449µg/ml, and the no toxicity against brine shrimp (LD\(_{50}\) 3423.156). Furthermore, no toxicity in all extracts was observed. The present study is the first evaluation regarding the characterization of *E. aegyptiaca* and its safety, and the results demonstrate its antioxidant potential and suggest its safe therapeutic use. The results suggest that methanol extract is a rich source of phytochemicals and exhibits highest amount of and total phenolic, flavonoid content and significant antioxidant activity and it has no cytotoxic activity. *E. aegyptiaca* plant can be regarded as a promising Source of naturally occurring potential antioxidants.

**Keywords:** *Euphorbia aegyptiaca*, Sudan, Antioxidant, Total phenolic, total flavonoid, Cytotoxicity.

**INTRODUCTION**

Researches in the field of plant antioxidant focus on antioxidant agents that can protect biological components from oxidative damage \([1]\). Dietary intake of phenolic compounds correlates with reduced coronary heart disease, cancer mortality and protective in many health-related properties, such as antioxidant, anticancer, antiviral and anti-inflammatory activities \([2, 3, 4]\). On the other hand, concern about the safety of the commonly used synthetic antioxidants such as butylated hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ) has led to increased interest in plant antioxidants \([5]\).

The genus *Euphorbia* asperata (Euphorbiaceae) is considered as a large family of flowering plants comprising around 300 genera with 7,500 species \([6]\). *E. aegyptiaca* known locally in Sudan as *umm lebaina* or *malhaine*. The whole plant is used against scorpion bites. In Sudanes traditional medicine as well as for treatment of inflammatory conditions like rheumatoid arthritis, conjunctivitis and dermatitis \([7]\). *Euphorbia* species contain phytochemical constituents like flavonoids, coumarins, terpenoids, lignans and alkaloids \([8,9]\).

To date, to the best of our knowledge there has been no report on the quantitative determination of total phenolic and flavonoid contents and cytotoxic activity of *E. aegyptiaca*. The aim of the present study was to screen the phytochemicals and to assess the antioxidant activity; total phenolic and flavonoid contents and cytotoxic activity of *E. aegyptiaca*. 

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\(\text{IC}_{50}\): concentration that inhibits 50% of the growth of the test organism. 

\(\text{LD}_{50}\): lethal concentration that kills 50% of the test organisms.

GAE: gallic acid equivalent. 

QE: quercetin equivalent. 

BHA: butylated hydroxyanisole. 

TBHQ: tertiary butylhydroquinone.
MATERIAL AND METHODS

Sample collection

Whole plant of was collected in the White Nile state, Sudan, during February 2016. The identity of the plant was confirmed at the Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan and the voucher specimens were deposited in the herbarium. The plant was dried for 15 days under the shade, then pulverized by mechanical grinder and stored in air-tight containers till use.

Chemicals

The chemicals and reagents used were of analytical grade. n-Hexane, Chloroform, Methanol, Dimethyl sulfoxide (DMSO), Gallic acid, ascorbic acid, aluminum chloride, sodium acetate and sodium carbonate were purchased from SDFC, India. Ethanol LR, was obtained from Duksan, South Korea. 2,2Dithiopyridine-1-picryl-hydrazyl (DPPH) and Quercetin were purchased from Sigma-Aldrich, UK. Foline-Ciocalteu reagent was purchased from CDH, India.

Apparatus

Grinder (Disk Mill Model, FFC-15, China), Sensitive Balance (Rad wag, As 220/c/2, Poland), Soxhlet apparatus, heat mantel, Rotary evaporator (Heidolph OB2000 Heidolph VV2000, Germany), Ultraviolet (UV/vis-s-240 (PC) S, Double beam) Spectrophotometer (Shimadzu Japan).

Preparation of extracts

Five hundred grams of plant material was powdered by a grinder and stored in air-tight containers. The prepared sample was mixed with few milliliters of methanol, chloroform and hexane successively in a Soxhlet apparatus. The percentage yield of the extracts was determined based on dry weight (d.w).

Storage of specimens

Whole plant of the species was deposited in the herbarium. The plant was kept in the refrigerator at 5°C until further analysis.

Determination of total phenolics content

The Folin Ciocalteu reagent was used for analysis of total phenolic content in plant extracts was determined [14]. Briefly, Test sample of each extract was prepared in methanol and the concentration of 1 mg/ml was used in the analysis. 1ml of the extract was mixed with 1 ml of Folin Ciocalteu reagent. The solution was kept at 25°C for 5-8 min before adding 4 ml of sodium carbonate solution 7.5 % and adjusting the volume to 16 ml with water. After 2 h, the absorbance was measured at 765 nm against the blank. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. Gallic acid was used as standard for the calibration curve. The content of phenols in the extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

Preparation of extracts

Five hundred grams were extracted successively with 2L n-hexane, chloroform and methanol successively in a Soxhlet apparatus. The extracts were filtered using Whatman filter paper no. 1, and the filtrates were concentrated to dryness using a rotary evaporator. The percentage yield of the extracts was determined based on dry weight (d.w). The samples were kept in the refrigerator at 5°C until further analysis.

Antioxidant activity

DPPH radical scavenging assay:

The DPPH radical scavenging assay was performed according to the method of Shimada et al., (1992) [13], with some modification. In 96-wells plate, the test samples were allowed to react with 2, 2-Di (4-tert-octylyphenyl)-1-picryl-hydrazylstable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as (300μM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at λ: 517nm using multiple reader spectrophotometer. Percentage radical scavenging activity of samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

IC50 Calculations:

The IC50 test material, which possesses 50% inhibition of free radicals of all the extracts and their fractions, was determined by monitoring the effect of different concentrations ranging from 0.5-0.0035mg/ml. The IC50 of the extracts and their fractions were calculated using EZ-Fit Enzyme Kinetic Program (Perrella Scientific Inc, U. S.A.).
95% confidence intervals by analyzing the data on a computer loaded with a “Finney Program.” The concentration at which it could kill 50% larvae (LD$_{50}$) was determined. LD$_{50}$ values below 200ppm are generally considered as significant [17, 18, and 19].

**Statistical analysis:**
Data were presented as means ± S.D. Statistical analysis of assay results were performed using the Microsoft Excel program 2013 and Finney Program.

**RESULTS AND DISCUSSION**

**Percentage Yield of Extract**

The plant material was extracted successively by the Soxhlet extraction method using n-hexane, chloroform and methanol. The results showed the highest yield of methanol (9.461%), followed by chloroform (1.902%) and then n-hexane (1.433%) as seen in Figure 1.

**Chemical Constituents:**

The phytochemical screening of three successive extracts prepared with n-hexane, Chloroform and Methanol, of *E. aegytiaca* plant revealed the presence of various secondary metabolites: tannins, flavonoids, coumarins, saponins, sterols, terpenes and alkaloids. Anthraquinones and Cyanogenic glycoside were not detected. Phytochemical constituents of the *E. aegytiaca* plant have been depicted in Table 1.

| No. | Plant Constituents | Tests/reagents used | Name of Extracts |
|-----|--------------------|---------------------|-----------------|
|     |                    |                     | n-Hexane | Chloroform | Methanol |
| 1   | Tannins            | Ferric chloride test| -        | -          | +++      |
| 2   | Flavonoids         | ALCL: 1% test       | +        | -          | ++       |
|     |                    | KOH 1% test         | +        | +          | ++       |
|     |                    | Shinoda’s test      | +        | +          | +++      |
| 3   | Coumarins          | NH$_3$ 10%          | -        | -          | +        |
| 4   | Saponins           | Froth Test          | -        | -          | +        |
| 5   | Sterols            | Salkowski’s Test    | -        | -          | +        |
| 6   | Terpenes           | Liebermann Test     | -        | -          | +        |
| 7   | Anthraquinones     | Borntrager’s test   | -        | -          | -        |
| 8   | Alkaloids          | Mayer’s reagent     | +        | +          | +        |
| 9   | Cyanogenic glycosides | Guignard test   | -        | -          | -        |

Key: + = Trace, ++ = Moderate, +++ = High, - = Negative.

**Total phenolic and total flavonoid contents**

The methanol extract showed the highest level of total phenolic contents (173.49±2.427 mg of GAE/g) and flavonoid content (239.53±7.90 mg QE/g), Table 2. Plants with high phenolics and flavonoids contents possess remarkable antioxidant activity due to their redox properties and chemical structures.

**Table 1:** Preliminary phytochemical screening of *E. aegytiaca*.

| No. | Extract | Total Phenolic content (mg of GAE/g of extract) | Total Flavonoid content (mg of QE/g of extract) |
|-----|---------|-----------------------------------------------|-----------------------------------------------|
| 1   | n-Hexane | 15.823±0.348                                  | 55.072±4.56                                   |
| 2   | Chloroform | 18.50±0.0483                                | 26.087±13.69                                   |
| 3   | Methanol | 173.49±2.427                                 | 239.53±7.90                                   |

Key: GAE= Gallic acid equivalent, QE= Quercetin equivalent, Values are expressed as means of triplicate determinations ± SD.
The free radical scavenging activity of E. aegyptiaca extracts were n-hexane (0%), chloroform (23%) but methanol showed the highest activity (89%) with least IC\(_{50}\) 0.0449 μg/ml. The results were reported Table 3.

Cytotoxic activity:

Cytotoxicity study of E. aegyptiaca extracts was conducted by brine shrimp lethality test. The results (Table 4), showed that LD\(_{50}\) Values above 1000 μg/ml were regarded as non-toxic [23].

Table 4. Cytotoxicity of E. aegyptiaca extract on (brine shrimp) by Finney probity analysis (model).

| No. | Extract   | LD\(_{50}\)     | Result        |
|-----|-----------|-----------------|---------------|
| 1   | n-Hexane  | 37095928695.2   | No-Toxic      |
| 2   | Chloroform| 12204.187       | No-Toxic      |
| 3   | Methanol  | 3423.156        | No-Toxic      |

Key: >1000: non-toxic LD\(_{50}\)= Lethal Concentration calculated by Probit analysis, D. J. Jon Finny.

CONCLUSION

It is proved that the methanol extract of E. aegyptiaca has the highest antioxidant activity, total phenolic and flavonoid contents. Furthermore, have no toxic effect. Therefore, it can be stated that E. aegyptiaca plant can be regarded as a promising candidate of natural antioxidant compounds with high values. These results might be helpful for treating diseases related to oxidative processes. The results of our investigations is a good starting points for Fractionation, isolation, purification and monitoring of activities of constituent according to their antioxidant effect. Identification and characterization of the chemical structure of these phenolics and flavonoids of the methanolic extract of E. aegyptiaca, Could be a remarkable addition to the global database.

REFERENCES

1. Zhang HY, Yang DP, Tang GY. Multipotent antioxidants: from screening to design. Drug Discovery Today; 2006; 11(15-16):749-54.
2. Halliwell, B. Dietary polyphenols: Good, bad, or indifferent for your health? Cardiovascular Research, 2007; 73:341–347.
3. Kubola, J., & Siriamornpun, S. Phenolic contents and antioxidant activities of bitter gourd (Momordica charantia L.) leaf, stem and fruit fraction extracts in vitro. Food Chemistry, 2008; 110:881–890.
4. Mohsen, S. M., & Ammar, A. S. M. Total phenolic contents and antioxidant activity of corn tassel extracts. Food Chemistry, 2009; 112:595-598.

5. F. Heshmati-Afshar, A. Delazar, H. Nazemiye, S. Esmeei and S. B. Moghadam, "Comparison of the total phenol, flavonoid contents and antioxidant activity of methanolic extracts of Artemisia spicigera and A. splendens growing in Iran," Pharmaceutical Sciences, 2012; 18(3):165-170.

6. Rahman AH MM and Akter M. Taxonomy and Medicinal Uses of Euphorbiaceae (Spurge) Family of Rajshahi, Bangladesh. Res. Plant Sci, 2013; 1(3):74-80.

7. Marium A. Abo-dola, Mohamed F. Lutfi. Anti-inflammatory activity of Euphorbia aegyptica extract in rats. International Journal of Health Sciences, Qassim University; 2016; 10. 1.

8. Liu ZJ, Li ZL, Bai J, Meng DL, Li N, Pei YH, Zhao F, Hua HM. Anti-inflammatory diterpenoids from the roots of Euphorbia ebracteolata. J Nat Prod, 2014; 77(4):792–9.

9. Llanes-Coronel DS, Gáméz-Díaz LY, Suarez-Quintero LP, Páez LJ, Torres F, Echeverri F, Ponte-Sucre A, Patiño PJ, Trujillo-Vargas CM. New promising Euphorbiaeae extracts with activity in human lymphocytes from primary cell cultures. Immunopharmacol Immunotoxicol, 2011; 33(2):279–90.

10. Harbone JB. Phytochemical methods: a guide to modern techniques of plant analysis. Champon and Hall Ltd, 1984, 49-188.

11. Sofowora A. Medicinal Plants and Traditional Medicines in Africa. Chichester John, Willey & Sons New York, 1993, 256.

12. Martinez A, Marcha fitoquimica. In Manual de Prácticas de Farmacognosia y Fitoquímica: 1999. 1. st edition.

13. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidant properties of xanthan on the abutioxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural Food Chemistry. 1992; 40:945-948.

14. Chun OK, Kim DO, Lee CY. Superoxide radical scavenging activity of the major polyphenols in fresh plums. J Agric Food Chem 2005; 53:8067-72.

15. D. Marinova, F. Ribarova, M. Atanassova, J. Univ, Chem. Tech. Met. (Sofia), 2005; 40(3):255-260.

16. Mayer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E and McLaughlin, J.L. Brine shrimp: a convenient bioassay for active plant constituents. Planta Medica, 1982; 45:31–34.

17. Kupahan, S.M., P.S. Steyn, M.D. Grove, S.M. Horsefield and S.W. Meitner. Tumor inhibitors, xxxv Myrsine saponin. The active principle of Myrsine africana. J. Med. Chem., 1969; 12:167-169.

18. Ma, W.W., J.E. Anderson, C.J. Chang, D.L. Smith and J.L. McLaughlin. Majorenolide and majorynolide. A new pair of cytotoxic and pesticidal alkene-alkyne δ-lactones from Persia major. J. Nat. Pdts, 1989; 52:1265–1266.

19. Oladimeji, H.O, Nia, R and Essien, E. E. In-vitro Anti-Microbial and Brine-Shrimp Lethality Potential of the Leaves and Stem of Calotropsis procera (Ait). African Journal of Biomedical Research, 2006; 9:205 – 211.

20. McLaughlin JL, Rogers LL, Anderson JE. The use of biological assay to evaluate botanicals. Drug Inf J, 1998; 32:513-524.