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Original Research Article

Euphorbia milii: Phenolic contents, Cytotoxic, and Antioxidant Prospect

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ABSTRACT

The development of pharmacopeial, non-pharmacopeial, or synthetic medications has extensive relied on exploiting medicinal plants as they are a rich source of medicinal components. The present study focused on examining the cytotoxic, phenolic, and antioxidant prospects of *Euphorbia milii.* The whole dried plant was extracted using n-hexane, dichloromethane, and methanol to produce the organic extracts. Total phenolic contents, cytotoxic contents, and antioxidant activities were measured using the Folin–Ciocalteu reagent method, brine shrimp lethality bioassay, and DPPH radical scavenging assay, respectively. Anthraquinones, saponins, cardiac glycosides, and flavonoids were identified by phytochemical analysis. The highest phenolic content (276.41 ± 64 mg GAE/g dry extract wt) was observed in the methanol extract. Significant ($P<0.04$) antioxidant potential was exhibited by the methanol extract (78.8% inhibition with IC_{50} value of 35.71µg/mL). Methanol extract showed notable cytotoxicity at the highest dose level in the brine shrimp lethality test, with an LD_{50} of 471.05 (µg/mL). The study found considerable antioxidant and cytotoxic properties in *Euphorbia milii*. Further study is advised to identify the secondary metabolites responsible for the biological activities described.

Keywords: *Euporbia milii*, Total phenolics, Antioxidant, Cytotoxic.

Introduction

Many plants contain enough extractable secondary metabolites in their tissues to be employed in treating a variety of diseases. Plants have been used for pharmacological effects since the dawn of human civilization. Bioactive compounds have been generated in significant quantities by medicinal plants. Research on these compounds has led to the development of pharmaceuticals with biological properties that fight disease and infection.[1] According to data from the WHO, more than 75% of the world's population uses herbal medicine.[2]

The method and equipments used for representing, synthesizing, and isolating natural compounds have seen significant advancement over time. Recent advancements in the study of secondary metabolites may be helpful in this area. According to a literature review, natural compounds originating from plants are essential for creating a reliable and plentiful source of medicines.[3] One of the key characteristics of natural products is their structural variety, which offers researchers vast opportunities to find new leading structures with superior pharmacological activities. According to an analysis of the literature, only 10% of plants have been studied in this way. Because of this, there is an excellent opportunity for researchers to find novel secondary metabolites with significant biological applications.

The plant family "Euphorbiaceae" belongs to *Phylum "Anthophyta*" Euphorbiaceae" is a huge family comprising of almost 326 genera and about 9000 species.

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Plants from this family are distributed all over the world. It occurs mainly in the tropics, with most species in the Indo-Malayan region. Almost 25 genera of Euphorbiaceae exist in Pakistan. The genus "*Euphorbia"* holds nearly two thousand species. The discrepancy within this genus is surprising, as it contains both low-growing garden weeds called "spurges" and gigantic, cactus-like succulents. The majority of Euphorbia succulents are present in temperate climates.

"*Euphorbia milii*" is an upright succulent. It is a woody succulent subshrub or shrub growing to 1.8 m (5 ft 11 in) tall, with densely spiny stems. The straight, slender spines, up to 3 cm (1.2 in) long, help it scramble over other plants. The fleshy, green leaves are found mainly on new growth. It has yellow flowers that appear at some point in the summer.[4] It has been reported that numerous Euphorbiaceae plants, teas, and fresh latex are being employed in alternative folk remedies. Bhuvaneshwar et al. in a study, described that *E. tirucalli* is used as a traditional medicine to combat various ailments, including rheumatism, asthma, arthritis, neuralgia, warts, cough, cancer, and gonorrhea.[5]

It has been demonstrated that free radicals have an impact on human health by speeding up the development of a number of chronic illnesses, such as diabetes, hypertension, cancer, heart disease, and other degenerative disorders.[6] The body's metabolic function generates free radicals. Consuming antioxidants can assist our body in lessening the negative impacts of free radicals. In recent years, there has been a huge interest in utilizing antioxidants to stop the harmful effects of free radicals on the human body. The use of antioxidants derived from natural sources is preferred over those derived from semi-synthetic sources.[7]

The present study is therefore aimed at determining the phenolic content, antioxidant activity, and phytochemical screening of *E. milii* extracts.

Materials and Methods

Collection of *Euphorbia milii*:

Euphorbia milii was collected from the botanical garden of B.Z.U. Mulan on 15 July 2021. Prof. Dr. Zaffrullah identified the plant

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as *Euphorbia milii.* The specimen voucher # 38FCV1 was deposited in the herbarium Department of Pure and Applied Biology, B.Z. U. Multan.

Extraction of *Euphorbia milii*:
For effective extraction, whole plant material was kept under the shade for drying for 15 days. After drying, it was grinded and weighed. The extraction of *E. milii* was carried out by successive maceration. Powdered material (500 g) was macerated with 2.25 Liter of n-hexane. The mixture was mixed after some time and then homogenized in the ultrasonic bath to obtain the greatest extraction possible. After 24 hours, this combination underwent filtration. The same technique was used to macerate the marc with n-hexane once more. Following the third filtration of the extract, the marc was similarly extracted with methanol and dichloromethane. A rotary evaporator was used to concentrate the solvent extracts. Extracts were weighed and assigned with codes as EMH, EMD, and EMM for n-hexane, methanol and dichloromethane extract, respectively.

Preliminary phytochemical analysis
Detection of various secondary metabolites was carried out by using standard tests. Detection of cardiac glycosides was carried out by using the Keller Kilini test.[8] Borntrager's test was employed for anthraquinones glycosides.[9] Similarly detection of saponins and alkaloids was performed by using standard methods.[10,11]

Determination of total phenolics
phenolic contents of the plant extracts were determined using the Folin-Ciocalteu reagent described by Singleton and Rossi. Plant extracts were taken in different test tubes. 5 mL Folin-Ciocalteu reagent was added to each tube. 4 mL of 7.5% sodium carbonate (Na_2CO_3) was added to each tube after 5 minutes. The sample was kept at room temperature for 3 hours. The absorbance was measured at 765 nm using microplate reader spectrophotometers (Molecular devices, VERSAmax tunable, California, USA). The absorbance of each sample was taken thrice. The standard curve of gallic acid solution (10, 20, 40, 60, 80, and 100 ppm) was prepared using a similar procedure. The total phenolic content of plant extracts was stated as mg GAE/100 g extract sample.

Antioxidant activity
Antioxidant potential of the plant extracts was evaluated by free radical scavenging using DPPH method. Free radical scavenging action of plant extracts on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical was studied to evaluate the antioxidant potential. Antioxidant evaluation was carried out by method proposed by Akowuah et al. in year 2005.
Aliquots of 200 µL of sample extracts were taken in test tube and 0.8 mL of methanol was added. 0.1mM DPPH methanol solution was added to each test tube. The mixture was shaken and the test tubes were kept for one hour in the dark. The control was prepared by mixing 1 mL methanol and 2 mL of DPPH. The absorbance of each sample was measured at 517 nm with the help of microplate reader spectrophotometers. Each reading was taken thrice. Percentage of DPPH scavenging activity was calculated using the formula below.
% inhibition of DPPH = [Abs control –Abs sample / Abs control] x 100.

Brine-Shrimp lethality assay
Procedure: Sea salt (3.8 g) was dissolved per liter of distilled water and then filtered in order to make artificial sea water. Shrimp eggs covered with aluminum foil were kept in larger slot of tank. These tanks were filled with artificial water prepared as mentioned above. Shrimp eggs were hatched and matured in 48 hours at controlled temperature of 22-29ºC. Three replicates of each extract were prepared. For this purpose, 20 mg of each sample was dissolved in 2 mL of suitable organic solvent. Afterwards these were shifted to 500, 50 or 5 µL vials correspondingly. Organic solvent was allowed to evaporate at room temperature. Insoluble content was dissolved in dimethyl sulfoxide (DMSO). 50 µL/5mL of artificial sea water was also added to vials. 5 mL artificial sea water and ten shrimps/ vial were added after 48 hours of maturation of larvae, with the help of Pasteur pipette. Vials were kept under illumination. After 24 hours, with the help of a 3x magnifying glass; the number of surviving shrimps were counted and recorded. Recorded data was investigated by using software (Probit analysis) in order to find out LC_{50} and 95% confidence intervals values.

Statistical analysis
Data was statistically analyzed by using SPSS software by IBM. All readings were taken thrice.

Results and Discussion

Phytochemical screening
Chemical analyses is performed to assess and identify the components of a medication sample. These tests are extremely specific for any one component or broad for a certain class of compounds, such as alkaloids. In several tests, color or turbidity develops. While turbidity in the sample tube is compared with a reagent containing the test tube in cases of precipitation reactions, color should be matched with an actual specimen. These tests are mostly applicable to extracts and isolated components.[12]
The phytochemical profile of the plant was studied by using standard phytochemical screening methods. Result of detection of secondary metabolites is summarized in Table 1.

Total phenolic contents
The total phenolic contents in the examined plant extract using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent. The values obtained for the concentration of total phenols are expressed as mg of GA/g of extract. Result of total phenolic contents in plant extracts is given below in Table 2.

Antioxidant activity
Antioxidant potential of the plant extracts was evaluated by using DPPH free radical scavenging assay. Plant extracts showed concentration-dependent increase in radical scavenging capacity. EMM showed 78.8% inhibition with IC_{50} value of 35.71µg/mL, at highest concentration (100 µg/mL). Whereas the dichloromethane extract exhibited 71.82% inhibition at dose of 100 µg/mL with IC_{50} of 41.88 µg/mL. n-hexane extract exhibited non-significant antioxidant potential. Result of antioxidant activity of plant extracts is shown in Figure 1. Ascorbic acid was used as standard in DPPH assay.

Brine shrimp (Artemia salina) lethality bioassay
Many secondary metabolites are found to be toxic for shrimp larvae. The Brine-Shrimp lethality assay is one of the rapid, cost-effective techniques for evaluation and scrutinizing of bioactive natural producs.[14]
Plant extracts of *Euphorbia milii* were investigated for cytotoxic activity by employing Brine shrimp lethality test. Methanol extract demonstrated cytotoxicity at highest level of dose with LD_{50} 427.18 (µg/mL). Non-significant activity was shown by n-hexane and dichloromethane extract. Results are shown below in Table 3.

Table 1: Result of detection of secondary metabolites *in Euphorbia milii*

Plant Extract	Alkaloids	Anthraquinones	Saponins	Cardiac glycosides	Flavonoids
EMH	−	−	+	−	+
EMD	−	+	+	+	+
EMM	−	+	+	+	+

(+) = Present, (−) =Absent

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Table 2: Total phenolic contents of the plant extracts

Plant Extract	TPC (mg GAE/g dry extract wt)
EMH	04.67 ± 0.71
EMD	106.23 ± 0.41
EMM	276.41 ± 64

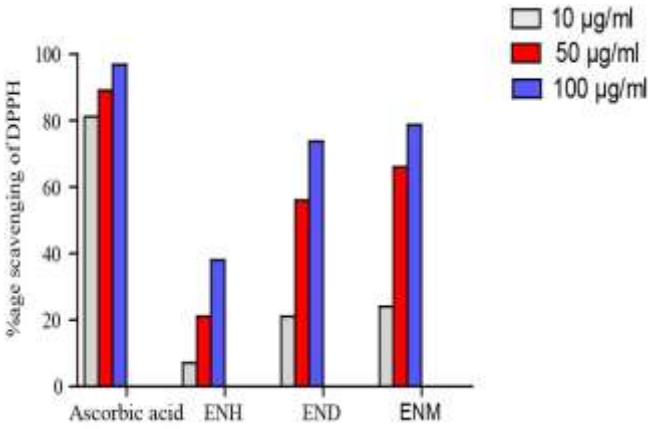

Figure 1: DPPH free radical scavenging activity of different extracts of *Euphorbia milii*.

Phenolic compounds are essential to plant constituents with redox properties responsible for antioxidant activity. The hydroxyl groups in plant extracts are responsible for facilitating free radical scavenging. Flavonoids are one of the essential secondary metabolites that hold considerable antioxidant and chelating actions. The substitution pattern of hydroxyl groups in the structure of any flavonoid contributes to its antioxidant action.[15,16]

Polyphenolic compounds from plant sources have been reported as potent antioxidants, attractants for insects, UV screens (flavonoids), and in the production of defense response chemicals. Various flavonoids play an essential role in the signal pathway. Phenolic compounds have a crucial role in human defense responses, such as antioxidants, anti-aging, anti-proliferative and anti-inflammatory actions.

The human body produces specific unstable molecules to react to various environmental and stress factors. Antioxidants also called "free–radical scavengers," that can avert or slow down harm to human body cells by free radicals.

Free radicals smash up the cell of any organism by damaging the DNA. These free radicals also produce oxidative stress by a series of reactions. Therefore, biological evaluation of crude extracts regarding antioxidant potential from plant origin is also increasing rapidly.[17,18]

The brine shrimp lethality assay symbolizes a fast, low-cost and straightforward bioassay for testing plant extracts bioactivity, which in most cases associates convincingly with cytotoxic and anti-tumor properties. It's a preliminary toxicity screen for further experiments on mammalian animal models. Several studies have shown that brine shrimp assay has been an excellent method for preliminary investigations of toxicity, for screening medicinal plants popularly used for several purposes, and for monitoring the isolation of a great variety of biologically active compounds.[19]

Table 3: Results of Brine shrimp (*Artemia salina*) lethality bioassay of n-hexane, dichloromethane and methanol extract of *Euphorbia milii*

Extract	Dose (µg/mL)	No. of shrimps	No. of survivors	LD50 (µg/mL)	Standard Drug	LD50 (µg/mL)
	1000	30	24			
ENH	100	30	28	42564.1	Etoposide	7.4625
	10	30	29			
EMD	1000	30	23	22563.3	Etoposide	7.4625
	100	30	26			
	10	30	29			
EMM	1000	30	02			
	100	30	25	471.5	Etoposide	7.4625
	10	30	28			

Conclusion

The study revealed that *Euphorbia milii* contains high phenolic content, most likely contributing to its antioxidant potential. The plant extract also exhibited significant cytotoxic potential at the highest tested dose. To the best of our knowledge, this is the first-ever report of the cytotoxic and antioxidant study of *Euphorbia milii*. Further investigation is suggested to isolate secondary metabolites responsible for reported biological activities.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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