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Original Research

Evaluation of Phenolic contents, Cytotoxic, and Antioxidant potential of *Euphorbia nerrifolia*

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Abstract

Objective: The researchers wanted to see how cytotoxic, phenolic, and antioxidant potential of *Euphorbia nerrifolia* were. Methods: The organic extracts were obtained by extraction of whole dried plant with n-hexane, dichloromethane and methanol. Phyto-chemical screening was carried out by using standard methods. Total phenolics contents were measured by using the Folin–CioEcalteu reagent method. Cytotoxic action was evaluated by brine shrimp lethality bioassay. DPPH radical assay was employed for antioxidant activity.
Results: Phytochemical investigations confirmed the presence of anthraquinones, saponins, cardiac glycosides and flavonoids. Highest phenolic content (276.41 ± 64 mg GAE/g dry extract wt) was observed in methanol extract. Significant antioxidant potential was exhibited by methanol extract by means of 78.8% inhibition with IC_{50} value of 35.71µg/ml). In brine shrimp lethality bioassay, methanol extract demonstrated prominent cytotoxicity at highest level of dose with LD_{50} 471.05. Conclusion: The study concluded that *Euphorbia nerrifolia* contains significant antioxidant and cytotoxic potential. Further investigation is suggested for isolation of secondary metabolites responsible for reported biological activities.

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Introduction

For very beginning of human civilization, plants had been effectively used for pharmacological actions. Medicinal plants had produced an extensive collection of bioactive molecules. Scientific studies of these molecules have resulted in production of medicinal substances with biological actions in opposition to infection and ailments [1]. Many higher plants hoard extractable secondary metabolites in adequate quantity to be utilized to cure a variety of disorders. Herbal medicines are used by more than seventy five percent of the word population as described by W.H.O. [2].

Over time there is considerable enhancement in the tactic and instrumentation employed for depiction, synthesis and isolation of natural products. Recent developments in this regard may be beneficial, resulting in advancement in exploration of secondary metabolites. Literature survey reports that plant derived natural products have vital role in production of reliable and affluent supply of drugs. Structural diversity of natural products is one of the prominent features, which provides the researchers many opportunities to discover novel lead structures with improved pharmacological actions. Plants and microorganism are major source of natural products. According to a literature survey, only ten percent of plants have been investigated in this regard. That's why researchers have immense potential to discover new secondary metabolites with significant biological potential. [3].

The plant family "Euphorbiaceae" belongs to *Phylum "Anthophyta"*. "Euphorbiaceae" is a very large family which comprises almost 326 genera and almost 9100 species. Plants from this family are distributed all over the world. The plants of this family are found in tropical regions, with the majority of the species in the Indo-Malayan region. Almost 25 genera of Euphorbiaceae exist in Pakistan the genus "*Euphorbia"* hold almost 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. Majority of Euphorbia succulent is present in temperate climates.

E. neriifolia is hairless upright branched succulent, may be a tree or shrub up to 22 ft or 1.9–4.6 m tall with incomprehensibly 5-angled branches [4]. The literature survey reports that several Euphorbiaceae plants, teas, and fresh latex are used these days in alternative folk medicines. Bhuvaneshwar et al. described that *E. neriifolia* is used as traditional medicine to combat various ailments including rheumatism, asthma, arthritis, neuralgia, warts, cough, cancer and gonorrhea. Latex of the plant is used as laxative and has been found effective for treating tumors and abdominal disturbance. Study also reveals that the latex of the same plant is employed as purgative, carminative, whooping cough, asthma and management of jaundice and kidney stones. Leaves of the plant have been used for handling abdominal pain, bronchitis, tumors and various inflammatory disorders. [5].

Free radicals have been proved to affect human health, by contributing to progression of different chronic ailments including diabetes, hypertension, cancer heart and other degenerative disorders [6]. Free radicals are produced via metabolic process in body. Antioxidants intake can help our body cut down the bad effects of free radicals. Remarkable interest in the use of antioxidants has been observed in recent years to prevent the unsafe effects of free radicals on humans. Preference is given to the use of antioxidants obtained from natural sources rather than from a semi-synthetic source [7].

Recent study was conducted to explore phytochemical screening, phenolic contents, and antioxidant action of various extract from *E. nerrifolia.* Outcome of this research work may add to the therapeutic potential of the selected plant.

Materials & Methods

The examination study was performed in pharmaceutical chemistry lab, Faculty of Pharmacy, B.Z.U. Multan. Detailed depiction of material & method is illustrated below.

Collection of *Euphorbia nerrifolia*

Euphorbia nerrifolia., was collected from forest park of Perrowal distric Khanewal. Prof. Dr. Zaffrullah recognized the plant such as *Euphorbia nerrifolia.* The sample voucher # 38FCV1 was placed in the herbarium of department of pure and applied biology, B.Z. U. Multan**.**

Extraction of *Euphorbia nerrifolia*

Whole plant material was dried for 15 days in the shade for optimal extraction. It was dried, then ground and weighed in a grinding mill. The *Euphorbia nerrifolia* was extracted using a series of macerations. An extraction container introduced a determined amount of n-hexane to 500 gm of powdered material. This mixture was agitated for a while before being homogenized in an ultrasonic bath to determine the effectiveness of the extraction. After 24 hours, the mixture was filtered. Then, following the same technique, marc was macerated again with n-hexane. The marc was extracted with dichloromethane and methanol in the same manner after the third filtering of the extract. The rotary evaporator was used to concentrate the solvent extracts. Weighing and assigning extracts.

Preliminary Phytochemical Analysis:

Compound tests are used in drug testing to analyze and identify ingredients. These tests are extremely specific for a single component or very broad for a group of compounds, such as alkaloids. Many tests produce turbidity or shading. Shading should be done in conjunction with a genuine example, and the turbidity in the example tube should be compared to the reagent-containing test tube alone if precipitation reactions occur. For the most part, these tests may be done to extricates and detached sections in the same way. [8].

Tests for cardiac glycosides

Recognition of cardiovascular glycosides was done by utilizing Keller Kilini test. 1g of ground drug under review was placed in test tube. Ten ml of 70% liquor was added to it. Then combination was bubbled for 2 minutes on water-shower and afterward sifted. Filtrate was weakened with twofold volume of refined water. Lead acetic acid derivation was added, trailed by filtration of arrangement that eliminated chlorophyll and different shades. The filtrate was exposed to extraction by utilizing 10ml of chloroform or carbon tetrachloride. Chloroform layer was isolated. It was vanished by utilizing china dish

over water shower. Scarcely any ml of 3.5% ferric chloride in frosty acidic was added to buildup to break up it. Later on, moved to test tube following couple of moments. Then, at that point, sulfuric corrosive was poured down what isolated as the second-rate layer. Heart glycosides were affirmed by showing up of light green tone at upper layer and Brown tone at interface on standing [9].

Test for anthraquinones glycosides

0.5g of drug was taken and extracted using ten ml of boiling water for 10 minutes. Later on, subjected to filtration. It was cooled, later on extracted by using ten ml of CCl_4. The CCl4 layer was removed from the test tube, and 5 mL of water was added. Following that, 5ml of weak ammonia solution was added and thoroughly mixed. As a result of the lack of color, no free anthraquinones were found (pink to cherry-red).

For testing of bound anthraquinones separate test was led. 0.4g of powdered drug was extricated utilizing ten ml FeCl3 arrangement and 7ml Hydrochloric Acid. It was warmed utilizing water shower for 15 mints. The arrangement was separated, and the filtrate was cooled. It was then separated with 10ml of CCL4. The carbon tetrachloride was isolated trailed by washing with 5ml of distils water. 5ml of dil. NH4 arrangement was added to it. No anthraquinone glycosides were uncovered as nonattendance of shading (extreme pink to cherry-red) in drug under study [10].

Saponin glycosides tests: 0.5g powdered medication was mixed with water. The presence of saponins was detected in dense foam [11].

Alkaloids tests: 3g of pummeling medication under research was ingested and briefly flooded with 10ml of dil. HCl. It was cooled, and the rubbish was allowed to settle. The supernatant fluid was transferred to another test tube. In 1ml of filtrate, 4 drops of Dragendorff's reagent were added. Turbidity was observed, indicating the presence of alkaloids.

Extras of the filtrate were made soluble by adding weaken smelling salts arrangement. It was moved to isolating channel, separated with 10 ml of chloroform with gentle shaking. Two layers were noticed. Chloroform layer (lower) was extricated with 10ml of dil.CH3COOH. The concentrate was similarly isolated into four test tubes. 2-5 droplets of Dragendorff's reagent, Wagner's reagent, Mayer's reagent was added to 3 test tubes, individually. Each cylinder was contrasted with untreated test tube, for assessment of turbidity or hasten, which reconfirmed the presence of alkaloids in the test. [12].

Determination of total phenolics

Polyphenolic compounds are significant plant constituents with redox properties responsible for antioxidant action. Presence of ploy-hydroxyl groups in secondary metabolites of plant extracts participate vital task in facilitating free radical scavenging action. Total phenolic contents of plant extracts were determined using Folin-Ciocalteu reagent as described by Singlaton and Rossi (1965). 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 few minutes. Sample was kept at room temperature for 3 hours. Absorbance was calculated at 766 nm using spectrophotometers. Absorbance of each sample was taken thrice. Standard curve of gallic acid solution was prepared using the parallel course of action. Total phenolic content of plant extracts was stated as mg GAE/100 g extract sample.

Antioxidant Activity

Because of its ease of use and low cost, DPPH is the predominant approach for determining antioxidant potential. Antioxidant prospective of plant extracts was evaluated by free radical scavenging using DPPH method. Free radical scavenging action of plant extracts on D.P.P.H. radical was studied to evaluate the antioxidant potential. Antioxidant evaluation was carried out by scheme recommend via Akowiuah *et al.* (2005) [13].

The control was prepared by mixing 1 ml methanol and 2 ml of DPPH. A test tube was filled with 200 µl of sample extracts and 0.8 ml of methanol. Then 0.1mM DPPH methanolic solution was poured to every test tube. Blend was shake well; test tubes were kept for one hour in dark. Each sample's absorbance was measured at 517 nm with the help of microplate reader spectrophotometers. Each reading was taken thrice. One hundredth of DPPH actions was premeditated by formula given below.

% Inhibition of DPPH = [Abs control –Abs sample / Abs control] x 100.

Brine-Shrimp Lethality Assay

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 products [14].

Procedure: 3.8 g of sea salt was dissolved /L of refined water and then filtered to make artificial sea water. Shrimp eggs covered with aluminum foil e larger slot of tank. These tanks were filled with artificial water prepared as mentioned above. Shrimp eggs were hatched and mature in 48 hours at controlled temperature of 22-29 ºC. 3 replicates of every extract were prepared. For this purpose, 20mg of each sample was dissolved in 2ml of suitable organic solvent. Afterwards these were shifted to 500µl, 50µl or 5µl vials correspondingly. Organic solvent was allowed to evaporate at room temperature Insoluble substance was added to DMSO. 5ml fake ocean water and ten shrimps/vial were added following 48 hours of development of hatchlings, with the assistance of Pasteur pipette. Vials were held under brightening. Following 24 hours, with the assistance of a 3x amplifying glass. A while later the figure of existing shrimps was counted and recorded. Recorded information was explored by utilizing programming (Probit examination) to figure out LC50 and 95% certainty stretches values.

Results and Discussion

Phytochemical screening

The plant's secondary metabolites profile was studied by using standard phytochemical screening methods. Result of detection of secondary metabolites is summarized in Table 1.

Total phenolic contents

Ciocalteu's. The total phenolic content of the chosen plant extracts was determined using Folin-reagent. The result is represented as, gallic acid equivalent. Result of

total phenolic contents in plant extracts is given below in table 2.

Antioxidant activity

The plant extricates' cancer prevention agent capability was assessed by utilizing DPPH free extremist searching examine. Both extracts demonstrated concentration-dependent increase in radical scavenging capacity. ENM 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:

Plant extracts of *Euphorbia nerrifolia*. were investigated for cytotoxic prospective by employing Brine shrimp lethality test. Methanol extract demonstrated cytotoxicity at peak level of dose with LD_{50} 427.18. Non-significant activity was shown by n-hexane and dichloromethane extract. Results are shown below in table below 3.

Discussion

Phenolic chemicals are significant plant ingredients having antioxidant activity due to their redox characteristics. Free radical scavenging is facilitated by the hydroxyl groups in plant extracts. Flavonoids are one of the important secondary metabolites which hold considerable antioxidant and chelating actions. Substitution pattern of hydroxyl groups in structure of any flavonoid contribute to its antioxidant action. [15].

Polyphenolic compounds from plant sources have been reported as potent antioxidants, attractants for insects, UV screens (flavonoids), and defense response chemicals. Various flavonoids play an important role in signal pathway. Phenolic compounds have a key role in human defense responses, such as antioxidants, anti-aging, anti-proliferative and anti-inflammatory actions. [16].

Human body produces certain unstable molecules as a reaction to various environmental and stress factors. Antioxidants, also called “free –radical scavengers, " are substances 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 represents a quick, low-cost, and simple bioassay for determining the bioactivity of plant extracts, which is usually associated with cytotoxic and anti-tumor activities. It's a preliminary toxicity test in preparation for more research on mammalian animal models. Several studies have demonstrated that the brine shrimp assay is a useful tool for preliminary toxicity assessments, screening medicinal plants commonly used for a variety of reasons, and monitoring the isolation of a wide range of biologically active chemicals. [19].

Conclusions

The study revealed that *Euphorbia nerrifolia*. contains high phenolic content, which is most likely contributing to its antioxidant potential. Plant extract also exhibited significant cytotoxic potential at highest tested dose. This is the first report of a cytotoxic and antioxidant investigation of *Euphorbia nerrifolia* that we are aware of. Further investigation is suggested to isolate secondary metabolites responsible for reported biological activities.

Conflict of interest

All authors declare that there is no conflict of interest.

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

Plant Extract	Alkaloids	Anthraquinones	Saponins	Cardiac glycosides	Flavonoids
ENH	−	−	+	−	+
END	−	+	+	+	+
ENM	−	+	+	+	+

(+) = Present **(−) =Absent**

Table 2. Total phenolic contents in the plant extracts (expressed in terms of gallic acid equivalent (mg of GA/g of extract)

Plant Extract	TPC (mg GAE/g dry extract)
ENH	04.67± 0.71
END	106.23 ± 0.41
ENM	276.41 ± 64

TPC: total phenol content GAE: Gallic acid equivalents

Table 3. Bioassay of n-hexane, dichloromethane, and methanol concentration of *Euphorbia nerrifolia* on Brine shrimp (Artemia salina) lethality.

Extracts	Dose (µg/ml)	No. of shrimps	No. of survivors	LD50 (µg/ml)	Standard Drug	LD50 (µg/ml)
ENH	1000	30	24	42564.1	Etoposide	7.4625
	100	30	28			
	10	30	29			
END	1000	30	23	22563.3	Etoposide	7.4625
	100	30	26			
	10	30	29			
ENM	1000	30	02	471.5	Etoposide	7.4625
	100	30	25			
	10	30	28			

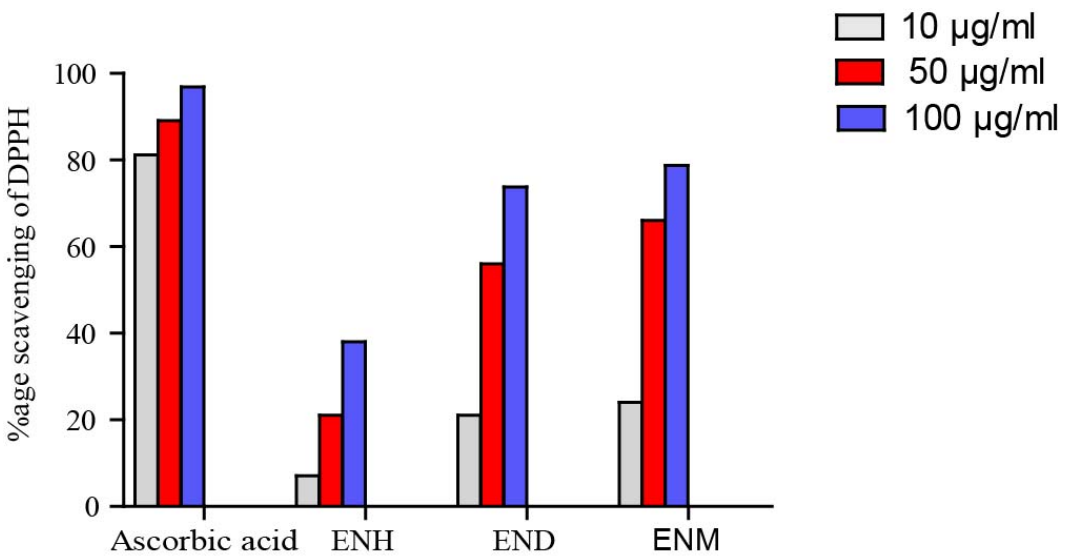

Figure 1: DPPH free radical scavenging activity of different extracts of *Euphorbia nerrifolia*.