Cytotoxic and Antioxidant Activities, and Phytochemical Analysis of Methanol Extract of *Euphorbia cotinifolia*

Muhammad Rashad [1*], Malik Saadullah [2]

Abstract

Background: Plants contain complex and structurally different phytochemicals. The popularity of herbal medicinal products for a variety of disorders in recent years has prompted researchers to study the therapeutic activities of several medicinal plants. The current study was carried out to investigate the cytotoxic and antioxidant activity, and various phytochemicals present in *Euphorbia cotinifolia.* **Material & Methods:** Crushed whole plant was used and extraction was performed by using methanol. Different physicochemical and identification tests were performed to determine the presence of phytochemicals, *folin-ciocalteu principle for phenolic compounds and standard quercetin method was used to determine total flavonoid contents*. FTIR for functional groups, DPPH method for antioxidant activity and MTT assay was used to evaluate the cytotoxic activity. **Results:** Methanol extract contained a significant amount of phenolics *(*62.81 ± 1.94 mg GAE/g) *and flavonoids* (33.75 ± 1.98 mg QE/g) *contents. FTIR spectrum* stipulated the occupancy of carboxylic acids, alkenes, and aromatic groups. Antioxidant activity was 82 % at 1000 µg/ml having IC_{50} value 18.61*±0.25* µg/ml as compared to standard (ascorbic acid). Cytotoxic activity showed percent inhibition 69.50 % and IC_{50} value 17.15 ±0.3 against standard (doxorubicin). **Conclusion:** It was concluded that *Euphorbia cotinifolia* contains phenolic and flavonoids compounds that are responsible for antioxidant and cytotoxic activity. It can be used for isolation of novel compound responsible for anticancer activity and other pharmacological actions.

Keywords: *Euphorbia cotinifolia*, Antioxidant, Total Phenolic contents, Total Flavonoids contents, cytotoxic activity.

Significance | This study determined the antioxidant, cytotoxic potential of *Euphorbia cotinifolia* and suggested its therapeutic applications.

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Introduction

Nature has bestowed upon us an abundance of medicinal plants utilized for herbal medicine to preserve human health. Medicinal plants, rich in bioactive constituents, contribute significantly to medication development (Wachtel-Galor & Benzie, 2011). Traditional herbal remedies have been used for centuries to treat infectious diseases (Maregesi et al., 2007). Increased awareness of the health risks of synthetic medicines has led to a surge in plant-based remedies globally (Anand et al., 2019). Yet, many therapeutic plants remain unexplored for their medicinal properties (Kaur et al., 2017).

The fight against free radicals, crucial in treating diseases like cancer, highlights the importance of antioxidant compounds found in plants (Allkin, 2017). The growing population, drug shortages, and side effects of allopathic drugs underscore the need for alternative treatments (Ekor, 2014). Euphorbiaceae family plants are known for their antioxidant compounds, making them

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promising candidates against diseases like cancer (Van Bavel, 2013).

Euphorbia cotinifolia, selected for its antioxidant potential, presents an opportunity for novel pharmacological research (Sofowora et al., 2013). This plant offers a wealth of components for drug synthesis, potentially effective against cancer and metabolic disorders (Aziz et al., 2018).

Materials And Methods

Collection and preparation of plant extract

Collection of *Euphorbia cotinifolia* was done from the main garden of model town park Lahore-Pakistan and identified by Professor Dr. Mansoor Ahmed, head of botany department the University of Agriculture, Faisalabad. After verification voucher specimen # EC-527 was allotted and the plant specimen was deposited in herbarium of botany department, University of Agriculture, Faisalabad. Plant was washed to remove filth, superfluous substances and it was dried in the shade for 30 days in order to get effective extracts. Dried plant was then grounded to get fine powder and weighed the final grounded powder. Extraction was performed through the process of cold maceration. 500 g of grounded plant was taken in glass bottle and 1000 mL of methanol was added to it. Bottle was put in ultrasonic baths to ensure optimum extraction. After 24 hours, the filtration carried out through whatman-filter paper (No.1). The similar process was done in triplicate. Rotating-evaporator was used to concentrate the extract at 37 °C under reduced pressure. The *Euphorbia cotinifolia* methanol extract was assigned the code ECM.

Preliminary phytochemical analysis:

Generalized test were used for the identification of group of components, but particular for a single ingredient (Cardiac glycosides, Saponins, and alkaloids etc). Typically, these tests include the development of turbidity or a change in colour. The resulting colour was compared to the reference, and the same procedure was employed for precipitation or turbidity production. Control was performed without the use of any drugs and only with the presence of reagent. These tests are applicable to both extracts and isolated components [10].

Estimation of total phenolic content

Evaluation of total phenols was conducted using the procedure [11] with some changes. 1mg/ml of stock solution was obtained by dissolving 4 mg of ECM in 4ml of methanol. In order to make normal gallic acid solution 5 mg gallic acid was used. Dissolve the gallic acid in 5 ml of methanol and then further diluted for making a serial concentration dilutions of 10, 20, 40, 80, 100 and 120 µg / mL) [12].

The impacts of the total phenolic were represented in terms of the equivalent gallic acid, as well as the quantity of phenols were measured using the calibration curves created from standard gallic acid curves (GAE/g extract) [13].

Estimation of total flavonoid content

The colorimetric aluminum-chloride process for determining total flavonoids was adopted [14]. To make the stock solution, weighed 4 mg of ECM and dissolved it in 4 ml of methanol. 200 µl of standard solution was mixed with 100 µl of each aluminium chloride and 1 M potassium acetate, and then diluted with 4.6 ml of distilled water. Standard quercetin solution was prepared at the ratio of 1 mg/ml and diluted to create serial dilutions (10, 20, 40, 80, 100 and 120 µg / ml). Both the solutions were kept for 30 mins at room temperature as an incubation time, and the absorbance was measured at 415 nm using UV / Vis spectrophotometer. The amount of flavonoids was measured against the calibration curve for quercetin and the results were measured according to the quercetin equivalent (mg quercetin / g extract) [15] [16, 17].

Fourier Transform Infrared Spectroscopy

FTIR fingerprinting was used to do qualitative analysis on crude powdered plant extract. A powdered sample of E. *cotinifolia* (1 mg) was merged with 100 mg of KBr and pulverised to get fine powder. This powder was transferred to a die, which was then squeezed by a hydraulic press to produce a disc. This disc yielded an FTIR spectrum in the 4000-400cm^{-1} IR band. The peaks representing different regions were compared to typical organic compound peaks [18, 19].

Determination of Antioxidant activity by DPPH

This assay was performed according to Queiroz et al. (2009). The scavenging ability to 1,1-diphenyl-2-picrylhydrazyl stable radicals (DPPH) of ECM demonstrated its antioxidant potential. Different concentrations of ECM (200 – 1000 µg /mL were prepared, took 100 µL from each concentration and mixed with 0.1 mM ethanol solution of DPPH (500 µL). After incubation at room temperature for 30 min the absorbance measurement was taken at 517 nm. Then, the ascorbic acid in different concentrations were used as standard to plot dose response curve and to draw linear regression equation (R^2). This experiment was executed thrice to get average percentage of inhibition. The low in absorbance refers to the high radical-scavenging action that may be calculated by using the following equation [20, 21].

$$(\%)\ \text{Inhibition} = \frac{\text{(Blank Absorbance– Sample Absorbance)}}{\text{Blank Absorbance}} \times 100$$

Anticancer assay (MTT assay)

MTT test was used to measure cytotoxic effects in 96-well microplates [22]. Human prostatic-cancerous cells (PC_3) were used to culture in a medium called DMEM (Dulbecco's Modified-Eagle's Medium), with which there is 5% of FBS (fetal-bovine serum) is used, and 100 IU/mL of antibiotic such as penicillin and 100 µg/mL of another drug such as streptomycin was used in 75 cm^2 flasks, that was kept in an incubator of 5% CO_2 at 37°C. The rapidly growing cancerous cells were harvested and counted with a special counter

Table 1: Qualitative phytochemical screening of *E. cotinifolia*

Chemical Constituents	Test Performed	Methanol Extract
Carbohydrates	Molisch Barfoed's Benedict's Fehling's	-
Glycosides	Legal's Keller-Killani's	+
Phenolics and tannins	Ferric-chloride Lead-acetate Gelatin	+
Alkaloids	Mayer's Hager's Wagner's	-
Sterols and triterpenoids	Libermann- Burchard	+
Flavonoids	Alkaline-reagent	+
Saponins	Foam test	-
Terpenes	Salkowski	+
Lipids	Fixed-oils and fats	+

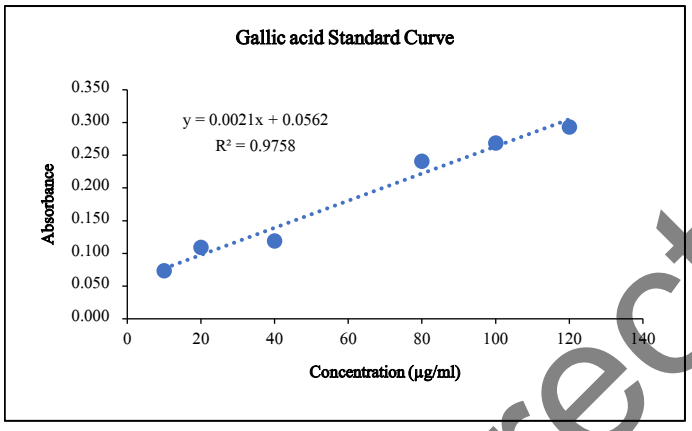

Figure 1: Gallic-acid standard curve

Table 2: Total phenolic-contents present in *E. cotinifolia*

Plant Extracts	Total phenolic (mg GAE/g) ± SD
ECM	62.81 ± 1.94
The values expressed are mean ± standard deviation (n=3)	

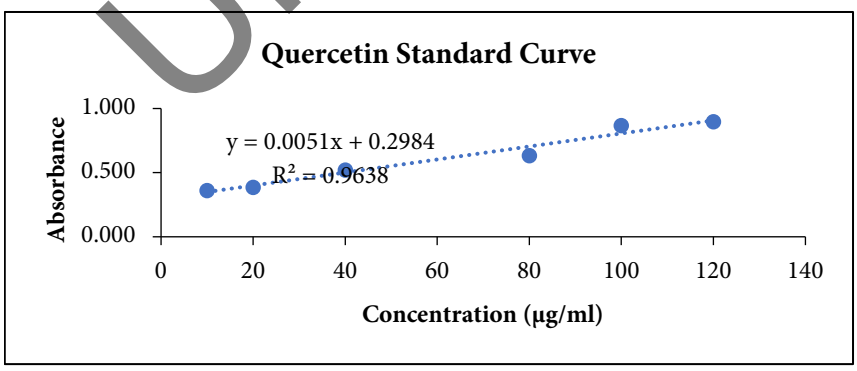

Figure 2: Quercetin standard calibration-curve

Table 3: Total flavonoid content (mg QE/g) ± SD

Extracts	Total flavonoid-contents (mg QE/g) ± SD
Methanol	33.75 ± 1.98
The values are indicated as mean ± standard deviation (n=3)	

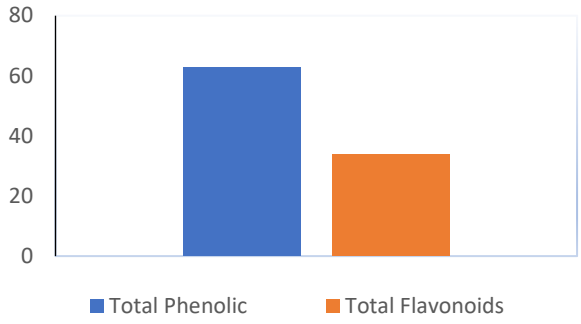

Figure 3: Comparison of total phenolic and flavonoid contents of ECM

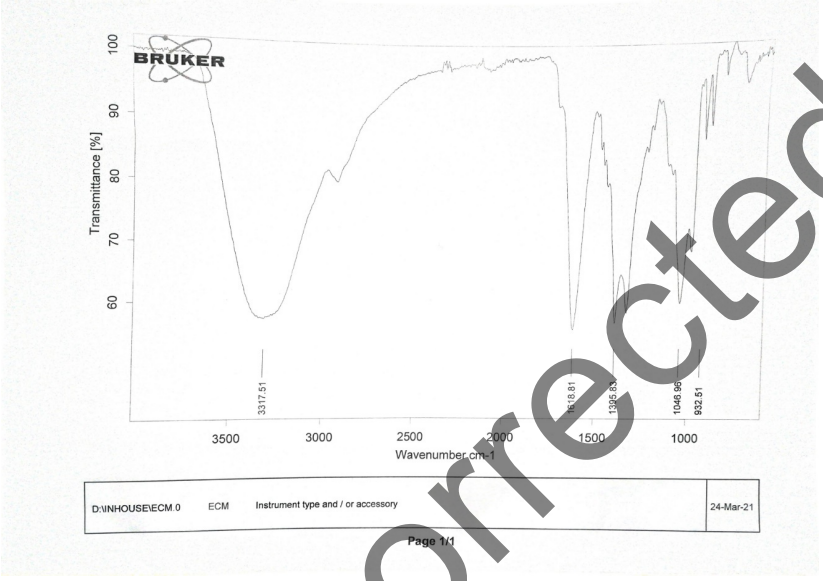

Figure 4: FTIR spectrum of ECM

Table 4: IC_{50} value of ascorbic acid (standard) and ECM

Sample	IC_{50} ± SD (µg/ml)
Ascorbic acid	11.37 ± 0.14
ECM	18.61 ± 0.25

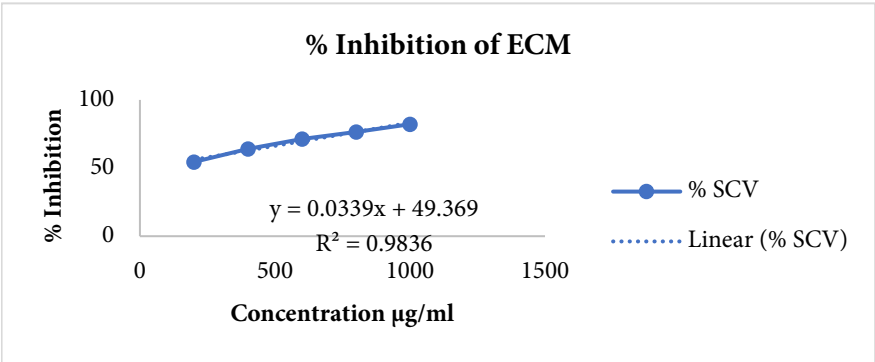

Figure 5: Percentage-inhibition of ECM

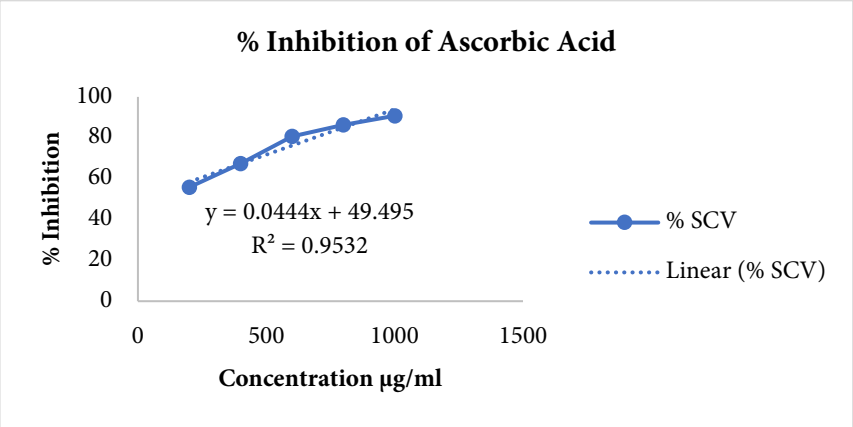

Figure 6: Percentage-inhibition of ascorbic acid

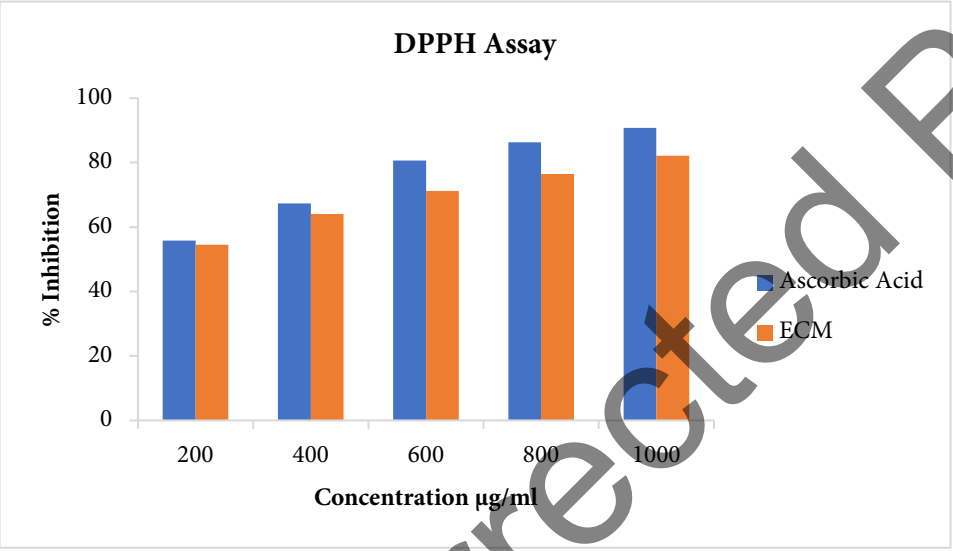

Figure 7: Comparison of DPPH free-radical scavenging activity of Ascorbic-acid and ECM

Table 5: Percent inhibition and IC_{50} value of ECM, and Doxorubicin at 50 µg/ml.

Sample	Concentration	% Inhibition	IC_{50} ± SD [µg/ml]
ECM	50 µg/ml	69.50	17.15 ±0.3
Standard (Doxorubicin)	50 µg/ml	89.45	0.92 ±0.2

called hemocytometer and then it was diluted with a specific medium. Cell culture having the concentration of 1 x 105 cells/mL was formed and subjected (100 µL/well) to 96-well plates. After the incubation period, the medium was discarded and 200 µL of newly prepared medium was introduced against the concentrations of compounds (1-30 µM). 48 hours after the incubation period, 200 µL MTT (0.5 mg/mL) was introduced to each well and furthermore incubated for 4 hours. 100 µL of DMSO was then introduced to each well [23, 24].

The power of MTT elimination was calculated by identifying the absorbance of each well at 570 nm by the use of micro plate reader. The cytotoxicity was calculated as the concentration having 50% growth inhibition (IC_{50}) for PC_3 [25].

Result

Phytochemical Analysis

Phytochemical analysis were performed by using different methods and identification of glycosides, carbohydrates, alkaloids, phenolics and tannins, flavonoids, sterols and triterpenoids, terpenes, saponins, and lipids were recorded as they are listed in *Table 1*.

Total Phenolic Content

Total phenolic contents were measured by equation obtained by standard Gallic-acid curve (*Figure 1*).

$Y = 0.0021X + 0.0562$, $R^2 = 0.9758$

The results obtained indicated that crude methanol extract exhibited the phenolic content GAE 62.81 mg / g, respectively (*Table 2*).

Total Flavonoid Contents

Total contents of flavonoid in crude methanol-extract of *E.cotinifolia* were measured by the equation of standard quercetin-curve termed as quercetin equivalent (*Figure 2*).

$Y = 0.0051X + 0.2984$, $R^2 = 0.9638$

According to obtained result, it was notices that total flavonoid contents in methanol extract of *E. cotinifolia* was (mg QE/g) 33.75, respectively (*Table 3*). A comparison of flavonoid-contents and phenolic-contents of ECM are visualized in *Figure 3*.

Fourier Transform Infrared Spectroscopy

In this study *E. cotinifolia* methanol extract was subjected to FTIR analysis and it showed the exhibition of biologically potent constituents. FTIR spectra showed the peaks at 3317.51, 1618.81, 1395.83, 1046.96 and 932.51 cm^{-1} stipulated the occupancy of functional groups, carboxylic acids (O–H stretch), alkenes (–C=C– stretch) and (=CH_2 bending), aromatics C-H stretch and C=C bending (*Figure 4*).

In-vitro antioxidant activity of *E. cotinifolia* by DPPH method

The percentage inhibition of ECM (*Figure 5*) was 82 % at 1000 µg/ml which reveal the IC_{50} value of 18.61± 0.25 µg/ml as compared to standard (Ascorbic acid) which showed percentage inhibition 90 % at 1000 µg/ml (*Figure 6*) and reveal the IC_{50} value 11.37± 0.14 µg/ml. Comparison of DPPH radical-scavenging potential of Ascorbic acid and ECM at different concentrations are visualized in *Figure 7* with IC_{50} value in *Table 4*.

Anticancer Assay (MTT assay)

Anticancer activity of ECM and ECDM was performed by MTT assay and the results obtained are summarized in *Table 5*.

Discussion

Herbal remedies have grown in popularity as a type of treatment. Despite the fact that traditional remedies and conventional pharmaceutical therapies have significant distinctions, herbal medicine may be evaluated for efficacy employing standard trial procedures. Several plant extracts have shown to be effective in treating particular ailments. Herbal medications do have dangers, despite the public misconception that all-natural therapies are fundamentally safe. Finally, we need to figure out which herbal treatments are more harmful than helpful for certain ailments. Because of the present prominence of herbal remedies, more study should be conducted in this field [26, 27]. Physicochemical and phytochemical analyses of plant extracts as a part of standardization is necessary to evaluate quality and concentration of active principle. We can evaluate the optimal dosage, performance, limitation, contraindication, and application of phytotherapeutic agents by the process of standardization [28].

Phytochemical analysis of this plant reveals abundant phenols, tannins, and terpenoids in methanol extract. The occurrence of these metabolites is the evidence of antioxidant activity. The major antioxidants or free radical scavengers are flavonoids and phenolic substances. Because these phenolic compounds were confirmed to be present in the extract [8], they may be responsible for E. *cotinifolia* strong antioxidant potential [29].. Saponins and glycosides, for example, have been shown to have anticancer effects due to their strong antioxidant activities [30, 31] against free radicals and metabolites produced from drugs metabolism and various chemical reactions taking place in the body.

The results indicated that *E. cotinifolia* is rich in bioactive compounds and can be used as a potential anti-oxidants source for food and drug-industries [32]. It can also be used to scavenge the ROS and free radicals assisted diseases such as neurodegenerative disorders and diabetes. The anti-cytotoxic potential is a measure of its use for cancer and to abrupt the irregular cell growth [30].

As a result, preliminary phytochemical investigations help in identifying chemical elements in plant extract, which may aid in quantitative assessment, as well as discovering the source of pharmacologically active chemical compounds.

Conclusion

It was concluded that *E. cotinifolia* possess a significant amount of secondary metabolites which possess antioxidant activity and anti-

cytotoxic activity. Thus, *E. cotinifolia* can be used for isolation of novel compound responsible for these pharmacological activities.

Author contribution

M.R. and M.S. wrote, drafted, reviewed and edited the paper.

Acknowledgment

None declared

Competing financial interests

The authors have no conflict of interest.

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