



## Antimicrobial activity and Phytochemical Screening of extracts of *Euphorbia cotinifolia*

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### Abstract

Plants are a natural resource of many complex and structurally diverse compounds. In the recent years, the acceptance of traditional herbal remedies for several diseases has led researchers to investigate the antimicrobial activity of medicinal plants. The present study was carried out to determine the content of phenolic compounds, flavonoids, alkaloids and tannins, comparing the antimicrobial spectrum of the various extracts of *Euphorbia cotinifolia* against different human pathogenic strains. The agar disc diffusion method was used to determine the antimicrobial properties of the extracts. Acetone and hexane extracts of *Euphorbia cotinifolia* showed potent antibacterial activity against *Shigella boydii* (mean diameter of growth inhibition zones was  $18.90 \pm 1.67$  mm and  $19.60 \pm 1.45$  mm), *Escherichia coli* ( $20.65 \pm 2.53$  mm and  $17.90 \pm 0.66$  mm) and *Salmonella typhimurium* ( $16.73 \pm 3.91$  mm and  $17.10 \pm 0.9$  mm). The present study ascertained the potency of *Euphorbia cotinifolia* as an antimicrobial agent.

**Keywords:** antimicrobial, phytochemical, medicinal, *euphorbia cotinifolia*

### 1. Introduction

Nature is endowed with a rich wealth of medicinal plants which are a source of herbal medicine for maintaining human health. Medicinal plants are a vast source of compounds which make significant contributions to drug development programme [1]. Plants are used medicinally in different countries for the treatment of various infectious diseases [2]. In recent years, there has been a growing interest in the use of traditional herbal remedies for the treatment of several diseases [3]. With increasing awareness of the health hazards and toxicity associated with the unsystematic use synthetic drugs and antibiotics, interest, in the use of plants and plant-based drugs revived throughout the world [4, 5]. Still there are a large number of medicinal plants that remain to be investigated, for their possible medicinal properties. It is therefore interesting to study these plants to substantiate the traditional medical knowledge, and to evaluate their promotional and curative applications. Plants contain bioactive constituents which are considered as secondary metabolites [6]. These bioactive chemicals are synthesised in all parts of the plant body i.e. stem, bark, leaves, flower, seeds and roots. The biochemical activity of these chemicals varies from one part to another. So, different solvents are used to screen the plant parts for extraction of various phytochemicals [7]. This paper focuses on the phytochemical investigation and antimicrobial activity of *Euphorbia cotinifolia*.

### 2. Botanical description

*Euphorbia cotinifolia* is an evergreen shrub or small trees with broad oval shaped leaves and woody stems. The plant has milky sap and tiny flowers [8]. The older leaves are dark burgundy and new foliage is bright red in color. The foliage dies back in winter. It is a perennial plant which reaches to

height of 10ft to 15ft. *Euphorbia cotinifolia* is also known as smoketree spurge, tropical smokebush or Caribbean copper plant. The stems are purplish gray with long petroleum which hold thin, slightly fleshy and ovate shaped leaves that are about 3 inches wide and 4 inches long. Small white flowers in loose panicles bloom at the branch ends in summer. Different species of *Euphorbia* possess antibacterial, anticancer, antifeedant, antiviral and analgesic activity. *Euphorbia cotinifolia* isopropyl alcohol extracts showed antibacterial activity against *S.aureus*, *E.coli*, *P.aeruginosa* [9]. Phytochemical analysis of methanol and ethyl acetate extracts of *Euphorbia cotinifolia* revealed the presence of flavonoids, terpenoids, tannins and steroids [10].

### 3. Materials and Methods

#### 3.1 Collection of Plant Material

Fresh leaf, stem and root samples of *Euphorbia cotinifolia* were collected from the college campus. Collected plant material was shade dried for 15-20 days and the leaves were separated from the stems. The dried material was ground into fine powder in a Wiley Mill.

#### 3.2 Preparation of plant extracts

The obtained dried powder was used to prepare extracts. Soxhlet extraction was carried out using 15gm of each sample coarsely powdered plant material with 200 ml of each solvent (acetone, petroleum ether, hexane, ethanol) for 24-48 hrs. Fractions were then oven dried at  $45-50^{\circ}\text{C}$  for 48hr and weighed for yield. The dried extracts (biocrude) obtained were collected in glass vials. Respective biocrudes were dried in rotary vacuum evaporator & yields were calculated after weighing.

### 3.3 Collection of microorganisms

Microbial pure cultures were obtained from MTCC (Microbial type culture collection, Chandigarh). The bacterial cultures were grown on nutrient agar medium.

### 3.4 Determination of total phenolic content

In the procedure, the extract of 1ml was mixed with 500ul of 50% Folin Ciocalteu reagent. After 2 minutes, 1.5ml of 20% Na<sub>2</sub>CO<sub>3</sub> was added and incubated at room temperature for 2 minutes. The final volume of the tubes was made up to 10ml with distilled and the tube was incubated for another 2 hours at room temperature. Absorbance was measured at 760nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram of dry material [11, 12, 13].

### 3.5 Determination of total flavonoid content

The flavonoids content was determined by aluminium trichloride method using catechin as reference compound [14]. A volume of 125µL of extract is added to 75µL of a 5% NaNO<sub>2</sub> solution. The mixture was allowed to stand for 6 min, then 150µL of aluminium trichloride (10%) was added and incubated for 5 min, followed by the addition of 750 µL of NaOH (1M). The final volume of the solution was adjusted to 2500µL with distilled water. After 15 min of incubation the mixture turned to pink and the absorbance was measured at 510 nm. Results were expressed as catechin equivalents (mg catechin/g dried extract).

### 3.6 Determination of total tannin content

The tannins were determined by Folin - Ciocalteu method [15, 16]. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu Phenol reagent, 1 ml of 35 % Na<sub>2</sub>CO<sub>3</sub> solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an

UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract.

### 3.7 Determination of total alkaloid content

The plant extract (1mg) was dissolved in dimethyl sulphoxide (DMSO), added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/g of Extract [17].

### 3.8 Antimicrobial activity

Antibacterial activity of extracts of *Euphorbia cotinifolia* was determined by disc diffusion method using Kirby-Bauer technique [18]. The bacterial strains were maintained on Nutrient Agar. Standardized inoculum was transferred and spread evenly on a nutrient agar plate. Sterile Whatmann No.1 filter paper discs impregnated with with 30µl of a solution of 30mg/ml were placed on nutrient agar plates and allowed to diffuse for one hour at 4<sup>o</sup>C. The plates were then incubated at 37<sup>o</sup>C for 24 hr. Disc containing 5% DMSO served as negative control and Ciprofloxacin (10µg), Amoxicillin (10µg) served as positive control. The zone of inhibition around each disc was measured for sensitivity or resistance. Diameters of the inhibition zones were measured.

## 4. Results

The dried powder of plant materials was extracted successively with acetone, petroleum ether, hexane and ethanol in a soxhlet extractor and concentrated. The percentage yield of the dried concentrated extract was recorded and is given in Table 1.

**Table 1:** Biocrude composition of *Euphorbia cotinifolia*

Plant part	Acetone Extract	Petroleum ether Extract	Hexane Extract	Ethanol Extract
Leaf, Stem	4.24±0.051	3.93±0.33	2.86±0.029	2.63±0.019
Root	4.07±0.068	3.14±0.045	2.54±0.056	1.51±0.038

Values are presented as mean ± standard deviation

The results of Phytochemical Analysis of *Euphorbia cotinifolia* are given in Table 2. The total phenolic content was high in Acetone Extract i.e. 105.68 mg GAE/gm followed by ethanol. The total flavonoid content of the four extracts was found highest in acetone extract i.e. 62.34 mg catechin/gm dried extract. The tannins contents was examined using Folin-Ciocalteu reagent expressed in terms of gallic acid equivalent. The highest concentration of tannins was measured 18.56 mg GAE/gm in hexane extracts. The alkaloid content was seen

maximum in acetone and ethanol extracts i.e. 26.35 and 24.69 mg of AE/g of extract respectively.

The antibacterial activity of plant extracts is shown in Table 3. The antibacterial efficacy of various solvent extracts namely petroleum ether, acetone, hexane and ethanol against Human Pathogenic bacteria showed varied level of inhibition. The activity of different extracts were compared with standard drugs Ciprofloxacin and Amoxicillin.

**Table 2:** Quantitative determination of Phytochemicals

<i>Euphorbia cotinifolia</i>	Alkaloids	Phenol	Flavonoids	Tannins
Petroleum ether	18.25±2.4	75.8±5.0	24.69±0.56	10.28±0.7
Acetone	26.35±2.0	105.68±4.8	62.34±2.65	12.56±0.2
Hexane	15.56±1.4	88.96±4.9	50.24±2.1	18.56±0.03
Ethanol	24.69±1.38	95.8±6.72	33.78±1.80	9.22±0.11

Values are presented as mean ± standard deviation.

**Table 3:** Antimicrobial activity of extracts of *Euphorbia cotinifolia*

Microorganism	Zone of inhibition(in mm)					
	Acetone	Hexane	Ethanol	Petroleum ether	Ciprofloxacin	Amoxicillin
<i>Shigella boydii</i>	18.90±1.67	19.60±1.45	14.63±1.09	16.68±1.22	23.26±2.01	24.13±1.64
<i>Escherichia coli</i>	20.65±2.53	17.90±0.66	18.34±1.32	19.52±2.90	22.14±2.64	23.64±0.82
<i>Salmonella typhimurium</i>	16.73±3.91	17.10±0.9	16.65±0.87	12.34±0.34	23.26±3.0	24.03±0.56

Values are presented as mean ± standard deviation.

Diameter of the zone of inhibition including diameter of disc 6mm.

## 5. Conclusion

The demonstrated antimicrobial activities in all the four extracts of *Euphorbia cotinifolia* have given a preface of their potentials as antimicrobial agents. This activity is due to presence of phenols, tannins, alkaloids and flavonoids. The organisms used in the research study were causative agents of diarrhoea and dysentery. The extracts of *Euphorbia cotinifolia* inhibited the growth of these microbes. *Euphorbia cotinifolia* plant has great potential as antimicrobial agent and it can be used in the treatment of infectious diseases caused by resistant microorganisms.

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## 7. References

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