

Evaluation of Antibacterial Activity of Plant Extracts of *Euphorbia thymifolia* Against Human Pathogenic Bacteria

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Abstract-

The present study was aimed at assessing the antimicrobial potential of various extracts of *Euphorbia thymifolia* against human pathogenic strains of *Salmonella typhimurium* and *Shigella boydii*. The antibacterial activity was determined by agar disc diffusion and broth microdilution techniques. The results showed that the growth of the organisms were inhibited by both acetone and hexane extracts of *Euphorbia thymifolia*. The acetone extracts showed significantly higher zones of inhibition. The minimum inhibitory concentration of the leaf and stem extracts were 0.078, 0.156, 0.312mg/ml. The phytochemical analysis was carried out for the different parts of the plant. The qualitative analysis showed that alkaloids, phenols and flavonoids were present in both acetone and hexane extracts. The result of the present study indicate that *Euphorbia thymifolia* has many medicinal values and can be widely studied to extract natural compounds which are beneficial to human beings.

Keywords –*Euphorbia thymifolia*, antibacterial, plant extracts, phytochemical, medicinal

I. INTRODUCTION

Medicinal properties of plants are a boon to human mankind. However, during last few centuries synthetic drugs have gained more importance but this trend is on the reverse. First and foremost reason is side effects caused by synthetic drugs. Not only this, with increased consumption of synthetic drugs pathogens have started developing resistance towards these drugs [1], [2]. A perfect alternative to this is increase in usage of medicinal plants. Plants are an important source of bioactive compounds as all medicines available in therapeutics are derived from natural products. Plants possess a wide range of bioactive molecules which are used to treat chronic and infectious diseases [3], [4]. A large number of medicinal plants are used in various formulations for the treatment of various diseases caused by microbes [5]. There are many advantages of using medicinal plants like plants are easily available and can be grown in abundance. Medicinal plants also increase the immunity of body by providing body natural nutrients [6].

Euphorbia thymifolia is a member of the Euphorbiaceae family. Family Euphorbiaceae is one of the largest families of flowering plants, composed of over 300 genera and 8,000 species. *Euphorbia thymifolia* is a monocious, prostrate, annual herb with branches up to 25 cm long, with numerous adventitious roots [7], [8]. The stems are with white latex. In India it is known by the common name Duddhi, Dudhiya, Chhoti-duddhi. Chhoti Duddhi, as the name indicates is a small plant containing milky latex in it. The plant is present in the wastelands, along roadsides in humid conditions, abandoned fields. The whole plant contains various phytoconstituents such as carotene, vitamin C, phenols, tannins, carbohydrates, glycosides, sterols, antioxidants etc. It also has various medicinal properties such as laxative, sedative, blood purifier, anti-viral, anti-inflammatory, anti-spasmodic, anti-fungal, anti-bacterial and diuretic properties. Latex of plant is also used to treat eye disorder and wounds [9], [10].

Classification

Kingdom: Plantae
Phylum: Magnoliophyta
Class: Angiospermae
Order: Malpighiales
Family: Euphorbiaceae
Genus: *Euphorbia*
Species: *Euphorbia thymifolia*

II. MATERIALS AND METHODS

A. Collection of Plant Material

The whole plant was collected from the vicinity of college campus. The plant parts of *Euphorbia thymifolia* were carefully separated, washed under tap water followed by sterilized distilled water and shade dried for three to four weeks. The dried material was ground into fine powder in a Wiley Mill.

B. Preparation of Solvent Extracts

The powdered sample thimbles were subjected to acetone extraction in a soxhlet apparatus for a minimum of 24hr per solvent. The acetone extract was then partitioned between n-hexane and aqueous ethanol in separatory funnel. All

fractions were then oven dried at 45-50°C for 48 hr are 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.

C. Bacterial cultures

The Bacterial cultures viz. *Salmonella typhimurium* (MTCC-3224) and *Shigella boydii* (MTCC-11947) were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), and Chandigarh, India. Both bacterial strains were maintained in viable state via inoculation on Nutrient Agar and overnight incubation at 37°C.

D. Qualitative Phytochemical Analysis

The Qualitative analysis of bioactive compounds for different extracts have been analysed in this study. All the extracts were checked for the presence of Alkaloids, Carbohydrates, Flavonoids, Phenols, Saponins, Tannins and Terpenoids using standard procedures [11-13].

Test for carbohydrates - To 2 ml of plant extract, 1 ml of Molisch reagent and 4 drops of concentrated sulphuric acid were added. Formation of purple or reddish ring indicates the presence of carbohydrates.

Test for tannins - To 1 ml of plant extract, 2 ml of 5 % ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for saponins -To 1 ml of plant extract, 5-10 ml of distilled water was added and shaken in a graduated cylinder for 15 min lengthwise. Formation of 1 cm layer of foam indicates the presence of saponin.

Test for flavonoids

- a) To 2 ml of plant extract 1 ml of 1N aqueous NaOH solution was added and observed for the formation of yellow-orange coloration.
- b) 2 ml of plant extract was treated with 4 drops of concentrated sulphuric acid and observed for the formation of orange color.

Test for alkaloids -To 2 ml of plant extract, 2 ml of concentrated hydrochloric acid was added. Then 3 drops of Mayer’s reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

Test for steroids - To 1 ml of plant extract, equal volume of chloroform and 3 drops of concentrated sulphuric acid were added. Formation of brown ring indicates the presence of steroids

Test for phenols - To 1 ml of the extract, 2 ml of distilled water followed by 5 drops of 10% ferric chloride was added. Formation of blue or green color indicates the presence of Phenols.

E. Determination of Antimicrobial activity

Disc agar diffusion technique was employed for antimicrobial activity [14]. Paper discs were impregnated with 30 µl of a solution of 30 mg/ml and the standard antibiotic ciprofloxacin and amoxicillin was used as a control for comparison. Disc containing 5% DMSO served as negative control. Standard antibiotic discs of ciprofloxacin and amoxicillin served as positive control. The plates were incubated and the zone of inhibition around each disc was measured for sensitivity or resistance. Diameters of the inhibition zones were measured. The antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the plant extract [15], [16].

F. Determination of Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was performed by broth micro dilution using 96 well microtiter plates. In each well of 96 well plate 50µl of Nutrient Broth was added. In 12th well 100µl of Nutrient Broth and in 11th well, 50µl Nutrient Broth with 10% DMSO was added. 50µl of the stock solution of each extract (concentration 200mg/ml) initially dissolved in 10% DMSO was added to the first well and twofold serial dilutions were performed using micropipette up till the 10th well. The obtained concentration range was from 10mg/ml to 0.020mg/ml. 0.5 McFarland standard broth inoculum was diluted to the ratio 1:100 and added to 1st – 11th well. The plates were incubated at 37°C for 18 h. After 18 h 50µl of a 0.01% solution of 2, 3, 5-triphenyl tetrazolium chloride (TTC) was added to the wells and plates were incubated for another one hour. In the presence of bacteria TTC is reduced to red color. The red color obtained indicates the viability of cells. The inhibition of growth can be noticed when the solution in the well remains clear after incubation with TTC [17], [18].

III. RESULTS AND DISCUSSION

The results of Phytochemical Analysis of *Euphorbia thymifolia* are given in Table 1. Phytochemical Screening revealed the presence of alkaloids, flavonoids and phenols for all extracts. Steroids and saponins were absent in acetone and hexane extracts.

Table I : Phytochemical Analysis of Extracts of *Euphorbia thymifolia*

S.No.	Constituents	Extracts	
		Acetone Extract	Hexane Extract
1.	Carbohydrates	+	+

2.	Tannins	+	+
3.	Saponins	+	-
4.	Flavonoids	+	+
5.	Alkaloids	+	+
6.	Steroids	-	-
7.	Phenols	+	+

The antimicrobial activities of *Euphorbia thymifolia* extracts assayed against human pathogenic strains was qualitatively and quantitatively assessed by evaluating the presence of inhibition zones, zone diameter and MIC values. All the tested extracts have shown significant antimicrobial activity against *Salmonella typhimurium* and *Shigella boydii*. The inhibition range was recorded between 16 to 24 for acetone and hexane extracts as shown in Table 2. The MIC values obtained for different plant extracts are shown in Table 3. It was ranging from 0.312mg/ml to 1.25mg/ml against different bacterial strains.

Table II : Antimicrobial activity of *Euphorbia thymifolia* by disc diffusion method

Human Pathogenic bacteria	Zone of Inhibition(in mm)					
	<i>Euphorbia thymifolia</i>				Standard antibiotics	
	Stems, Leaves (AE)	Roots(AE)	Stems, Leaves (HE)	Roots(HE)	Ciprofloxacin	Amoxicillin
<i>Salmonella typhimurium</i>	20±1.66	17±0.33	19±0.58	16±0.74	23±1.26	24±1.03
<i>Shigella boydii</i>	21±0.94	18±1.05	21±0.29	17±0.33	24±0.49	22±0.91

Values are presented as mean ± standard deviation.

AE-Acetone Extract, HE-Hexane Extract

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

Table III. Minimum inhibitory concentration (MIC) of plant extracts against human pathogenic bacteria

S.No.	Bacteria	MIC Values			
		Stems, Leaves (AE)	Roots(AE)	Stems, Leaves (HE)	Roots(HE)
1	<i>Salmonella typhimurium</i>	0.078mg/ml	0.312mg/ml	0.625mg/ml	0.625mg/ml
2	<i>Shigella boydii</i>	0.156mg/ml	1.25mg/ml	0.312mg/ml	1.25mg/ml

AE-Acetone Extract, HE-Hexane Extract

IV. CONCLUSION

Plants are a source of potent biochemical. They are beneficial therapeutic agents as they have received significant focus because of presence of various bioactive compounds, trace elements and other nutrients. *Euphorbia thymifolia* plant has great potential as antimicrobial agent and it can be used in the treatment of infectious diseases caused by resistant microorganisms. Also, further work can be carried on the isolation procedure for finding out the bioactive compounds responsible for the biological activity.

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