

Antibacterial activity of *Euphorbia Hirta* leaf extracts against some human bacterial pathogens

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Abstract

Euphorbia hirta powdered Medicinal plant material was extracted using 4 solvents DMSO, Methanol, Acetone, and Aqueous. The antibacterial activity of the plant leaf extract, the Disc diffusion method, and the Well diffusion method was used to assess the antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus*, human pathogens. Minimum inhibitory concentration (MIC) of DMSO extracts of leaves showed the lowest MIC against four tested microorganisms followed by methanol, acetone, and aqueous extracts. The lowest minimum inhibitory concentration (0.125mg/ml). Minimum bacterial concentration (MBC) of DMSO extracts of leaves showed the lowest MBC against all the tested microorganisms followed by methanol, acetone, and aqueous extracts. The lowest minimum bacterial concentration (0.25mg/ml) of *Euphorbia hirta* leaves.

Keywords: antibacterial activity, MIC, MBC, *Euphorbia hirta*

Introduction

In order to examine the antibacterial activity of medicinal plants, the development of microbial resistance to available antibiotics has led to resistance. As the therapeutic effectiveness of current antibiotics is being undermined by the emergence of multi-drug-resistant pathogens, antibiotic resistance has become a global concern. Natural products have infinite possibilities, either as pure compounds or as standardized plant extracts [1]. In the developing world, infectious diseases are significant causes of mortality and morbidity and account for about 50 percent of all deaths. Most of the pathogens that cause enteric infections have established resistance to the widely prescribed antibiotics that increase mortality. Hospitalization, and the duration of stay for most bacteria in the hospital [2]. Isolation of less susceptible bacteria to intermittent antibiotics and recovery in antibacterial therapy, resistant isolates are now a global concern. Several Infectious enteric bacteria, though not limited to *Staphylococcus spp*, *Proteus spp*, *E.coli*, *Pseudomonas spp*, *Shigella spp*, and *Salmonella spp*. Typically present as commensals, these bacteria have many virulent variables and colonise in a biofilm form, causing a number of intestinal and extraintestinal diseases [2]. *Euphorbia hirta* belongs to the family *Euphorbiaceae* [1]. *Euphorbia* is one of the most diverse genera in the family in the *Euphorbiaceae*. Members of the family and genus are sometimes referred to as spurge [3]. In Nigeria, it was a herb Used for female disorders, but more relevant in the treatment of respiratory disorders now Diseases, especially cough, bronchitis and asthma. It is used in India to treat Infestations of worms in children and dysentery. Jaundice, Gonorrhea, pimples, gonorrhoea Problems of metabolism, and tumours. The plant is also widely used against dysentery diarrhea and diarrhea, especially amoebic dysentery [4]. The plant has been commonly used historically as a treatment for skin problems, particularly intestinal parasitosis, amoebic diarrhea, Dysentery and ulcer gastrointestinal disorders. In order to prevent pathogenic

contamination, the latex of the plant is also used for warts and wounds. For the treatment of acute enteritis and dysentery, a violence decoction is used. The herb is also used for asthma, bronchitis, and hay fever, including bronchial disorders. *E. hirta* is well known for its biological functions, such as antibacterial, antifungal and anticancer antioxidants [5]. *Euphorbia* species have been used in the treatment of wounds in India ethnomedicine [6]. The plant leaves are also boiled jointly and singly or in multiple-dose. It is claimed that *E. hirta* extract alone or in a combination of gastrointestinal tract infections than conventional antibiotics (personal communication) [7]. So *E. hirta* has been proven to have wide pharmacological activities like antifungal, anti-allergic, anti-diarrheal, anti-inflammatory, Diuretic, antioxidant, anti-tumor, anti-diabetic and so on [8]. Recently their anti-tumor/anticancer properties and their activities against the Human Immunodeficiency Virus (HIV) have also been reported in *E. heterophylla* leaf [3]. The presence of sugar alkaloids, flavonoids, tannins, sterols, and triterpenoids in the whole plant was discovered by phytochemical examination of *Euphorbia hirta* [9]. Epidemiological trials have demonstrated that the existence of these phytochemicals has broad pharmacological properties [9]. The goal of this analysis is to determine the potential antibacterial activity. Extracts of *E. hirta* leaves against natural pathogenic bacteria [10]. For this work, the selected microbes were *Staphylococcus spp*. For human pathogens, this is one of the most common bacterial isolates [7].

Objectives of this study

- To Collection of *Euphorbia hirta* plant leaves.
- To determine the antibacterial activity of *Euphorbia hirta* plant leaf Ethanol extract, Methanol extract, Hexane extract, DMSO extract, Diethyl ether extract.
- To detection of *Euphorbia hirta* plant leaf extract Methanol extract, Hexane extract, DMSO extract,

Diethyl ether extract against some human bacterial pathogens.

Materials of this study

Methanol, DMSO, Acetone, Distilled Water, and all glassware used in this work were obtained from the Department of Microbiology, Annamalai University.

Microbial Cultures

The following test organism was obtained from the Department of Medical Microbiology, Annamalai University, and used for the present study.

- *Staphylococcus aureus* (Gram-positive).
- *Escherichia coli* (Gram-negative).
- *Pseudomonas aeruginosa* (Gram negative).
- *Klebsiella pneumonia* (Gram-negative).

The antibacterial activity of *Euphorbia hirta* plant leaf extract by measuring the zone of inhibition.

Plant Material

The fresh plant of *Euphorbia hirta* was collected from around Chidambaram, Cuddalore Dt, Tamil Nadu. *Euphorbia hirta* belongs to the family *Euphorbiaceae*.

Preparation of the extracts

They were washed with running tap water after collecting the leaves, and the leaves were shadow-dried for 20-30 days. They were separately made into powder after proper drying. 200g of powder sample was taken and immersed in a Soxhlet extractor in 500ml of methanol solvent (55-60° C) and held for extraction for approximately 8 cycles in 15-18 hrs. Due to their non-polar nature, DMSO (Dimethyl sulfoxide), Methanol, Acetone, and Aqueous were used in the solvents. The crude extract was extracted after the extraction of the powder sample, evaporated to dryness, the extracts were condensed by vacuum evaporated and preserved properly in a jar free of moisture. Methods for processing the dilution of crude extracts for antibacterial assays have been introduced. The extracts were dissolved in the same solvents as per mg/ml and further dilutions were rendered in the form of 100µg/ml, 50µg/ml, 75µg/ml and 25µg/ml.

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Preparation of Standard bacterial suspensions

The minimum number of viable microorganisms of *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, using surface viable counting methods of colony counts, per ml of stock suspension was found. About 10⁷- 10⁹ colonies can be identified by maintaining content experiment condition, each time a stock suspension can be prepared.

Disc diffusion method:

Antibacterial activity of the extracts was tested by the Disc-diffusion method (Kirby and Bauer method, 1996). The molten Muller Hinton agar media was poured into sterilizing Petri dishes separately which were labeled appropriately using an aseptic technique. Bacterial isolates were inoculated in nutrient broth tubes and incubated for 4 hours at 37° C. The batch of the culture was then adjusted to 0.5 McFarland turbidity standards. A moist swab with the broth culture of microorganisms was spread over the surface

of Muller Hinton Agar plate by mounting ball and froth in all directions uniformly. The excess inoculum was drained and the plate was allowed to dry for 5 minutes. The extracts of various solvents extracts were then placed on the surface of the inoculated Petri plate with sterile forceps and gently pressed to ensure contact with the inoculated agar surface. Ciprofloxacin (5 µg/disc) for bacteria were used as the positive control and 5ml DMSO was used as a negative control in those assay. The Petri dishes were then incubated at 37°C for 24 hours in an inverted position. The inhibition zones of microbes were observed and were measured in millimeters. A replicated study of each sample was conducted. Simultaneously control was also performed.

Well, the diffusion method

By using the method of agar well diffusion, the antimicrobial activity of *Euphorbia hirta* was established. It is one of the existing and economic methods of finding antibacterial activity. The molten Muller Hinton agar (Himedia) was poured into sterilizing Petri dishes separately which were labeled appropriately using an aseptic technique. Bacterial isolates were inoculated in nutrient broth tubes and incubated for 4 hours at 37° C. The cache of the culture was then adjusted to 0.5 McFarland turbidity standards. A moist swab with the broth culture of microorganisms was spread over the surface of Muller Hinton Agar plate by mounting ball and froth in all directions uniformly. The excess inoculum was drained and the plate was allowed to dry for 5 minutes. Four Wells were made in the plates (about 5.0mm diameter) using a sterile Cork borer and 100µg/ml, 50µg/ml, 75µg/ml, 25µg/ml of the extract was transferred into the well using a micropipette. The plates were allowed to stand for one hour for pre diffusion of the extract to occur and were incubated at 37°C for 24 hrs. The diameter of growth inhibition zones after incubation for 24 hrs using colony counts.

Minimum inhibitory concentration (MIC)

In the Muller Hinton Broth for bacteria, the minimum inhibitory concentration of plant leaves extracts was measured by the broth macro dilution process. To achieve a 128 mg/ml stock solution, the plant extracts were dissolved in 5 percent DMSO. To get a concentration between 0.125 and 0.06 mg/ml, 0.5ml of stock solution was mixed into 0.5ml of Muller Hinton Broth for bacteria. 50µl of the organisms' uniform suspension was transferred into each tube. The control tubes for bacteria were incubated for 24 hours at 37° C. After the macroscopic assessment, the lower concentration which shows no growth of the tested organism was determined as MIC.

Minimum Bacterial Concentration (MBC)

By plating 100µl of samples from each MIC assay into freshly prepared Muller-Hinton Agar for bacteria, the MBC of the extracts was determined. The MBC was reported as the lowest concentration of extracts which, during the incubation time, did not allow any noticeable growth of the bacterial colony on the suitable agar plate.

Results

The antibacterial activity of *Euphorbia hirta* was evaluated against some human pathogenic bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*) based on the disc diffusion method

and well diffusion method and based on the lower turbidity on the test tubes (MIC). Also, the minimum bactericidal concentration (MBC) was analyzed. Four different solvents viz., Aqueous, acetone, Methanol, and dimethyl sulfoxide (DMSO) were obtained for all the above parameters and given below detailed.

Antibacterial activity by disc diffusion method

The antibacterial efficacy was measured by the process of disc diffusion (Kirby Bauer). In this, three different concentrations of disc (mg/disc) were prepared viz., 50, 25, 12.5 mg/ml. *Euphorbia hirta* leaves DMSO extracts demonstrated greater antibacterial efficacy against all of the microorganisms studied followed by Acetone, methanol,

and aqueous extract. The DMSO extract (50 mg/ml) of *Euphorbia hirta* leaves showed maximum zone of inhibition (28 mm) against *Pseudomonas aeruginosa* followed by *Staphylococcus aureus* (21mm) *Escherichia coli* (20mm) and *Klebsiella pneumoniae* (18mm). It was followed by methanol, acetone and aqueous extracts showed a minimum zone of inhibition against all the tested bacteria. The positive control of ciprofloxacin (5µg) showed the zone of inhibition (33mm) against *Pseudomonas aeruginosa* followed by *Staphylococcus aureus* (30mm) *Escherichia coli* (29.5mm) and *Klebsiella pneumonia* (30mm). No zone of inhibition was seen in DMSO blind control. The results were given in Table -1.

Table 1: Antibacterial activity of leaf extracts of *Euphorbia hirta* (disc diffusion method)

The concentration of the leaf extracts of <i>Euphorbia hirta</i> (mg/ml) and zone of inhibition (mm)														Positive control
Microorganisms	DMSO			Methanol			Acetone			Aqueous				
	50.0	25.0	12.5	50.0	25.5	12.5	50	25.0	12.5	50.0	25.0	12.5		
<i>S. aureus</i>	28	24	21	23	20	16	24	21	17	21	18	15.3	25	
<i>E. coli</i>	27	24	22	25	21	18	23.5	20.5	18.3	22	17.6	15.8	22	
<i>P. aeruginosa</i>	20	21	19	21.6	19.7	16	20.4	17.8	15	19	16.8	15.0	34	
<i>K. pneumoniae</i>	23	18	16	22.4	20	17.6	21.4	19	16.7	20	17	14.2	33	

Antibacterial activity by well diffusion method

The antibacterial activity was assayed by the good diffusion method. In this, four different concentration of well (mg/well) were prepared viz., 100, 75, 50, 25 mg/ml. the DMSO extracts of *Euphorbia hirta* plant leaves showed higher antibacterial activity against all the tested microorganisms followed by methanol, acetone, and aqueous extract. The DMSO extract (100mg/ml) of

Euphorbia hirta leaves showed maximum zones of inhibition (47mm) *Pseudomonas aeruginosa* against and *Staphylococcus aureus* was followed by *Escherichia coli* and *Klebsiella pneumoniae* (30mm) was followed by methanol, acetone, and aqueous extracts showed minimum zone of inhibition against all the tested bacteria. The resulted were given in the Table. 2.

Table 2: Antibacterial activity of leaf extracts of *Euphorbia hirta* (Well diffusion method)

The concentration of the leaf extracts of <i>Euphorbia hirta</i> (mg/ml) and zone of inhibition (mm)																
Microorganisms	DMSO				Methanol				Acetone				Aqueous			
	100	75	50	25	100	75	50	25	100	75	50	25	100	50	75	25
<i>P. aeruginosa</i>	47	30	26	17	40	28	24	16	35	20	23.4	15.5	30	18	22	14
<i>S. aureus</i>	45	26	20	16	35	24	19	14	30	23.2	18.5	13.2	25	22	17	12.2
<i>E. coli</i>	40	24	19	15	30	22	20	13	25	21.5	19	12.5	24	18	16	11.5
<i>K. pneumoniae</i>	30	23	18	14	25	20	18	12	24	19	16	11.5	23	17	10.5	9.5

Minimum inhibitory concentration (MIC)

The DMSO extracts of leaves showed the lowest MIC against all the tested microorganisms followed by methanol, acetone, and aqueous extracts. The lowest minimum inhibitory concentration (0.125mg/ml) of DMSO extract of *Euphorbia hirta* leaves was obtained against *Pseudomonas aeruginosa* and *Staphylococcus aureus* which was followed by *Klebsiella pneumoniae* and *Escherichia coli* and their respective values were 1mg/ml. the results were given in the table.3.

Table 3: Minimum inhibitory concentration of the leaf extracts of (*Euphorbia hirta* against bacteria)

Minimum inhibitory concentration (mg/ml)					
Microorganisms	DMSO	Methanol	Acetone	Aqueous	Positive control
<i>P. aeruginosa</i>	0.125	0.25	1	2	0.125
<i>S. aureus</i>	0.125	0.25	1	2	0.125
<i>E. coli</i>	1	2	4	4	1
<i>K. pneumoniae</i>	1	2	4	4	1

Minimum Bacterial concentration (MBC)

The DMSO extracts of leaves showed the lowest MBC against all the tested microorganisms followed by methanol, acetone, and aqueous extracts. The minimum bacterial

concentration (0.25mg/ml) of DMSO extract of *Euphorbia hirta* leaves was obtained against *Staphylococcus aureus* and *Pseudomonas aeruginosa* which was followed by *Klebsiella pneumoniae* and *Escherichia coli* and their respective values were 1mg/ml. the results were given in the Table- 4.

Table 4: Minimum bacterial concentration of the leaf extracts of (*Euphorbia hirta* against bacteria)

Minimum bacterial concentration (mg/ml)					
Microorganisms	DMSO	Methanol	Acetone	Aqueous	Positive control
<i>P. aeruginosa</i>	0.125	0.5	2	4	0.10
<i>S. aureus</i>	0.125	0.5	2	4	0.10
<i>E. coli</i>	2	4	8	16	2
<i>K. pneumoniae</i>	2	4	8	32	2

Discussion

In the present investigation, DMSO extracts of *Euphorbia hirta* showed the highest antibacterial activity against all four bacterial strains. The antibacterial activity was demonstrated to various degrees, with both strains and dose-

dependent activity. Till now researchers have worked on antibacterial activity study taking leaf extract of *Euphorbia hirta*. In the present study DMSO, methanol, acetone, and aqueous extracts were effective against all the strains used in the study. Moreover, we have seen that methanol and DMSO extracts were more potent against *Pseudomonas aeruginosa*, *Staphylococcus aureus*. The extracts used, in this investigation have displayed variable antibacterial activities probably due to the differences in the phytochemical constituents extracted by the different solvents used in our study.

Conclusion

Herbal medicine has become popular in healthcare. Medicinal plant science has recently acquired a revived emphasis. The primary explanation is that other treatment schemes, while effective, have many side effects that also lead to severe complications. The plant-based system of medicine that is natural does not pose this serious problem. The findings obtained from our results and also from other researchers concluded that the plant extract has great potential as antibacterial compounds against microorganisms and that they can be used in the treatment of infectious disease caused by resistant microorganisms. Our major focus in future studies is to isolate and characterize the bioactive compounds from these plants.

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