International Journal of Advanced Multidisciplinary Research
ISSN: 2393-8870
www.ijarm.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijamr Volume 10, Issue 7 -2023

Research Article

DOI: http://dx.doi.org/10.22192/ijamr.2023.10.07.007

Studies on Preliminary Phytochemical Screening and *In vitro* Antibacterial Activities of *Euphorbia hirta*

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Keywords

Euphorbia hirta, methanol extract, Antibacterial activity, medicinal plant.

Abstract

Euphorbia hirta has been used for hundreds of years as a health promotion and treatment. The emergence of new infections, drug-resistant bacteria, high costs of synthetic drugs and their voracious side effects have led to an increase in the use of traditional medicine globally. Hence, an attempt has been made in this study to examine the antibacterial activity of the methanol extract of *Euphorbia hirta.* The methanol extract was prepared by Soxhlet extraction. A number of bioactive compounds have been revealed from *Euphorbia hirta* that are found to be responsible for the pharmacological potential. Thus, the preliminary phytochemical screening was done to reveal the bioactive constituents of *E. hirta*. Qualitative phytochemical studies exhibited the presence of saponins, flavonoids, terpenoids, alkaloids, tannins, anthroquinones, steroids and glycosides compounds. There are two different pathogenic organisms (*Staphylococcus aureus* and *Escherichia coli)*selected against which the antibacterial activity of the extracts was determined. The result of the antibacterial activity showed that them ethanol extract of *E.hirta* showed the strongest antibacterial activity against the bacterial strains(*Staphylococcus aureus* and *Escherichia coli)* by the agar well diffusion technique. Concentrations of 50mg/ml, 100mg/ml, 200mg/ml and 400mg/ml were made. The result showed that 400mg/ml of the extract consistently showed the highest zone of inhibitions with the highest antibacterial effect on *Staphylococcus aureus*. The findings show that *E. hirta* extracts have promising antibacterial potential.

Introduction

Many medicinal plants are used in modern medicine, where they occupy a very significant place as raw material for important drugs. The plants used in traditional systems of medicine in pharmaceutical houses are collected from wild sources[1].More than 80% of the world's population relies on medications produced from these medicinal herbs for primary health care, according to the World Health Organization (WHO). The usage of medicinal plants as a source of disease alleviation may be dated back to the beginning of recorded history[2,3,4]. These phytomedicines are both effective and eco-friendly. Phytochemicals are a diverse group of bioactive compounds produced by plants [5]. The *Euphorbia hirta* is a member of the *Euphorbiaceae* family and belongs to the genus *Euphorbia*[6, 7]. It is commonly known as Amman pacharisi in Tamil and Asthma weed, hairy spurge, garden spurge, snakeweed in English. In the Philippines it is used traditionally as a curative for dengue fever and known as tawa tawa. It is a small annual perennial herb with milky toxic latex.

The plant*E. hirta* is a common herb among traditional herbal medicine practitioners. It's also known as a pill-bearing purge and asthma herb. The plant's stem is thin and reddish, with yellowish bristles covering it, especially in the immature parts of *E. hirta* [8, 9]. The search for a more natural, alternative source of disease therapies has become a global priority. *E. hirta* is a tropical plant that can be found in both urban and rural areas, and if its antimicrobial properties are confirmed, it will mean a more wholesome, easily accessible phytotherapeutic alternative to more conventional pharmaceutical antimicrobials, thereby contributing to the improvement of the healthcare system in our environment[10].Thus, the main aim of this work was to detect the various bioactive components present in *E. hirta*, and subsequently determine the antibacterial activity of *E. hirta* against *Staphylococcus aureus* and *Escherichia coli*, thereby proving its use as a safe and potent agent.

Materials and Methods

The whole plant, *Euphorbia hirta,* was collected, identified and authenticated by Dr. V. Balasubramanian, Senior Agriculture Officer at Coimbatorein Tamil Nadu(Figure.1). The methanol extract was successively prepared by the hot continuous percolation method in a 1:10 (w/v) ratio by Soxhlet extraction and concentrated. Then it was subjected to dryness to yield crude residue. The extract was kept at 4°C in the refrigerator until it was needed. This residue was employed for antibacterial evaluation. Preliminary phytochemical analysis of *Euphorbia hirta* was done by standard procedures [11-13]. Qualitative analysis was carried out to identify the phytochemical compounds in the plant. For the evaluation, the pure bacterial strains of *Staphylococcus aureus* and *Escherichia coli*were used. The isolated organisms were properly identified by their colonial morphology, Gram-stain result, microscopic appearance, and biochemical reactions.

The antibacterial susceptibility of plant extracts to *Staphylococcus aureus* and *Escherichia coli* bacteria was determined using an agar well diffusion experiment. The test organisms were inoculated onto Muller Hinton agar medium using a standardized bacterial suspension. On each plate, a sterile borer with a diameter of 5mm was used to make wells, with two of the wells put aside as controls. With the help of plastic pipettes, about 50µl of different concentrations (400mg/ml, 200mg/ml, 100mg/ml, and 50mg/ml) were put into the wells. As a positive control, the antibiotic Ciprofloxacin was used. The plates were incubated at 37°C for 24 hours and zone of inhibition were measured. A ruler was used to measure and record the diameter of the inhibitory zones. All tests were done in triplicate. All result values were expressed as mean ± standard deviation (SD). The student t-test was used for comparison of mean differences between and among groups, respectively, at a 95% confidence interval. The P-value $\leq$ 0.05 is considered statistically significant.

Results and Discussion

The preliminary phytochemical screening of *E. hirta* revealed that the methanolic extract contains flavonoids, saponins, terpenoids, alkaloids, tannins, anthroquinones, steroids and glycosides compounds (Table No. 1). The results of antibacterial activity show the comparisons of the Zone of inhibitions (ZIDs) of the methanol extract and control drug against the test organisms(Table No. 2). From the table, 400mg/ml of the extract constantly showed the highest ZIDs on all the tested microorganisms, with the highest antimicrobial effect on *Staphylococcus aureus* and *Escherichia coli*. The 50 mg/ml concentration showed the lowest ZIDs. There was also no growth seen in culture plates containing the positive control. A statistically significant difference ($p<0.0001$) was shown in the mean ZIDs of individual test organisms with respect to different extract concentrations and the control antibiotic. The results revealed that the crude extract of methanol is potently antibacterial against the test organism. The preliminary evaluation emphasises further research to describe the bioactive compounds involved for their antibacterial activity and to evaluate their other pharmacological activities of the *Euphorbia hirta*[14].

Figure. 1. *Euphorbia hirta* plant

Figure. 2.Phytochemical analysis of Methanolic extract of *E. hirta*

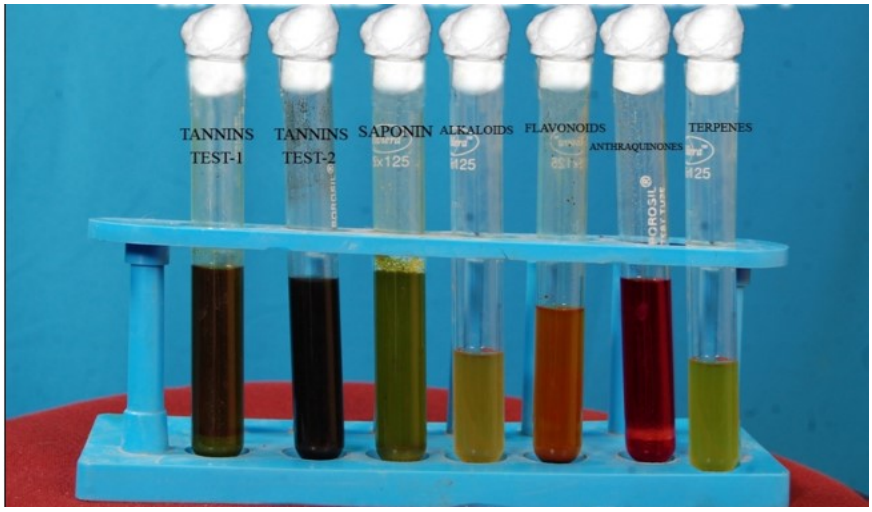

Table 1: Preliminary Phytochemical analysis of Methanolic extract of *E. hirta*

S. No	Test	Methonolic Extract of *E. hirta*
1	Tannins Test -1 Test -1	 Positive Positive
2	Alkaloids	Positive
3	Saponins	Positive
4	Flavonoids	Positive
5	Terpenes	Positive
6	Anthroquinones	Positive
7	Glycosides	Positive
8	Steriods	Positive

Table No2: Result of Zone of Inhibition of Antibacterial activity of Methanolic extracts of *E. hirta*

S. No	Name of the bacteria	Extract concentration	Mean Zone of Inhibition (mm)
1	***Staphylococcus aureus***	400 mg/ml	17.02 ± 0.24
		200 mg/ml	15.02 ± 0.20
		100 mg/ml	11.22 ± 0.10
		50 mg/ml	9.31 ± 0.40
		Positive control	21.25 ± 0.27
	p-value		<0.0001
2	***Escherichia coli***	400 mg/ml	15.10 ± 0.10
		200 mg/ml	13.14 ± 0.08
		100 mg/ml	10.26 ± 0.03
		50 mg/ml	7. 22 ± 0.12
		Positive control	19.48 ± 0.13
	p-value		<0.0001

The plant *E. hirta* is traditionally used to combat various ailments like amoebiasis, diarrhoea, peptic ulcers, heartburn, skin and mucous membranes and respiratory disorders[15]. Antibacterial activities of them ethanol crude extracts of *E. hirta* have been evaluated in the present research work. The *in vitro* study of antibacterial plant extracts might be a first step toward the invention of novel medicines. The antibacterial properties of *E. hirta* against a range of microorganisms were assessed in this study (*Staphylococcus aureus* and *Escherichia coli*) and the results obtained are consistent with the reports available. *E. hirta*'s efficacy on bacterial isolates has been studied by several researchers, with promising results [16-18]. The extract was also found to be most effective on gram-positive bacteria *Staphylococcus aureus* and gram-negative *Escherichia coli*[19, 20].

Conclusion

It has been concluded that the methanolic extracts of the *Euphorbia hirta* plant showed significant antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* by the agar well diffusion method. The large inhibition zones shown by the extract against *Staphylococcus aureus* justify the plant used in the treatment of sores, boils, and open wounds. Our work has supported other works that the *Euphorbia hirta* plant (Asthma weed) has antibacterial activities that increase with the increase in concentration. *E. hirta* is an herb that is often used by traditional healers to cure a variety of ailments. *E. hirta* extracts have promising antibacterial potentials. The presence of important phytoconstituents like saponins, flavonoids, terpenoids, alkaloids, tannins, anthroquinones, steroids and glycosides compounds could be responsible for the antibacterial properties (Figure.2). The crude extracts of *E. hirta* could inhibit Gram positive as well as Gram negative bacteria, indicating that the active ingredients are broad-spectrum compounds. However, thorough research needs to be done in order to recognise the phytoconstituents responsible for the antibacterial activity before being used for the development of any drugs.

Acknowledgments

The authors would like to thank Dr. V. Balasubramanian, Senior Agriculture Officer at Coimbatore on the TNAU campus, for the botanical verification and authenticating the plant material.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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DOI:10.22192/ijamr.2023.10.07.007	

How to cite this article:
Venkatajothi Ramarao, Seethalakshmi Illanchezian. (2023). Studies on Preliminary Phytochemical Screening and *In vitro* Antibacterial Activities of *Euphorbia hirta*. Int. J. Adv. Multidiscip. Res. 10(7): 57-62.
DOI: http://dx.doi.org/10.22192/ijamr.2023.10.07.007