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INVESTIGATION OF ANTIMICROBIAL ACTIVITY OF *EUPHORBIA HIRTA* LEAVES

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ABSTRACT

In India, folklore medicinal herbs have many traditional applications for the treatment of ailments of various bacterial and fungal infections. The authentication of such claim is essential which can be established by a scientific researcher. So, the aim of present research to investigate the antimicrobial activity of *Euphorbia hirta* leaves. The petroleum ether, methanolic and aqueous extracts of leaves of *Euphorbia hirta* belonging to family Euphorbiaceae were isolated for the antimicrobial activity by employing agar well diffusion method across a few selected organisms like *B. subtilis, E. coli, S. aureus* and *S. cerevisiae*. The phytochemical analysis of petroleum ether, methanolic and aqueous extracts revealed the presence of tannins, related polyphenols, terpenes, anthocyanins, alcohols, steroids like ß-sitosterol and ß-amyrin. The antimicrobial activities due to phytoconstituents were assured by the absence or presence of zones of inhibition and by calculating the MIC values. The results concluded that antimicrobial activity of all the extracts were revealed from moderate to significant in contrast to standard.

KEYWORDS*: Antimicrobial activity, B. subtilis, E. coli, Euphorbia hirta, Staphylococcus aureus, S. cerevisiae.*

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INTRODUCTION

In the past decades, natural products play a vital role in the cure of diseases and a lot of plants with antimicrobial activity have been investigated by scientists throughout the globe. Infectious diseases are the key factors in the increase of death rate worldwide[1]. Drug therapy has a profound influence on the health statistics all over the world[2]. Herbal medicines are the most ancient form of the health care known to humankind. The word herbs used as herb medicine, also known as botanical medicines or phytotherapy or phytomedicine, means a plant or plant part that is used to make medicines, food flavors or aromatics oils for soaps and perfumes. A herb can be a leaf, flower, stem, fruit, bark or any other part of the plant used for medicinal properties[3-5]. The plant *Euphorbia hirta* belongs to family Euphorbiaceae. The plant is commonly called as Dudhi (ver: Hindi). It is an annual herb and native to tropical countries[6], has cylindrical stem often reddish in color and especially younger parts are enveloped with yellowish bristly hairs. The greenish or reddish leaves (5 cm long) have oppositely arranged lanceolate and tiny dense round clusters of flowers like appearance. The green flowers constitute the inflorescence type which is a peculiar feature of the euphorbias. When the leaves are cut, the stem yields milky or white juice[7-8]. The plant contains tannins, related polyphenols, terpenes, anthocyanins, alkaloids, steroids like ß-sito-sterol, ß-amyrin and glycosides. The plant extracts are employed in the cure of respiratory tract inflammations and asthma[9]. It is also employed in the management of chronic bronchitis, cough, other pulmonary complications in Mauritius[10] and also employed as ear drops and in the regimen of boils, sore and in supporting wound healing. It is extensively employed in Angola in contrast to dysentery and diarrhea, chiefly amoebic dysentery[11]. The leaves are used to treat various diseases as antipyretic, carminative, diuretic, purgative etc. Our work is directed to investigate antimicrobial activity of *Euphorbia hirta* leaves. Since there is no scientific study to substantiate the traditional claim on antimicrobial activity of the plant, the present study is being taken up.

MATERIALS AND METHODS

Collection and Authentication of Plant

The leaves of the *Euphorbia hirta* (Figure I) were collected from market and dried in the shade for about two weeks. The shade dried materials were powdered. Fresh leaves were identified and authenticated (Ref. no. Voucher specimen-T/102/2008) by Botanist Mr. Radhashyam Kharol, R.G. Maha Vidyalaya, Mandsaur, Madhya Pradesh (M.P.), India.

Collection of Organism

The organisms like *Bacillus subtilis (B. subtilis), Staphylococcus aureus (S. aureus)* as gram positive bacteria; *Escherichia coli (E. coli)* as gram negative bacteria; *and Saccharomyces cerevisiae (S. cerevisiae)* as a fungi were collected from Department of Pharmacognosy, B.R. Nahata College of Pharmacy and Research Center (B.R.N.C.P.R.C), Mandsaur, 458001, M.P., India.

Figure 1
Photograph of leaves with different parts of *Euphorbia hirta* plant.

Preparation of Plant Extracts

The powder of shade dried leaves (100g) was loaded into Soxhlet apparatus and extraction was done by Soxhlet extraction process (continuous hot percolation) using petroleum ether (60-80˚C), methanol and water separately. The extraction was executed for 72 h, using three solvents. The crude extracts were collected and made solvent free by distillation. The percentage yield was determined by all selective successive extraction of *E. hirta* leaf with respect to dry weight. All the extracts were kept in desiccator for further study.

Preliminary Phytochemical Analysis of Extracts

The petroleum ether, methanol, and aqueous extracts were investigated to find out the various phytochemical constituents employing standard tests[12-14].

In-Vitro Antimicrobial Activity

The antimicrobial activity was analyzed by employing agar well diffusion method [15-16]. *S. aureus, E. coli, B. subtilis* bacterial strain *and S. cerevisiae* fugal strain were used for antimicrobial activity. After solidification of agar medium, sterile bores were used to make well of uniform diameter (6mm) and air tightened the molten agar to prevent the liberation of extract through bottom. Stock cultures were prepared in nutrient broth by inoculating one loop full of organisms and incubated for 25 h from old cultures. These were firmly swept over the agar (nutrient agar medium) plates using sterile cotton swab to make uniform culture lawns after overnight incubation. The extracts were freshly reconstituted with respective solvents such as petroleum ether, methanol and water and were taken in a concentration of 3mg/mL for anti-microbial activity and standard antibiotic-Ofloxacin (10µg) was used as positive antimicrobial control and was poured in each well and incubated for 18-24 h. The zone of inhibition was obtained with extracts of different solvents and the standard drug. The extracts were freshly reconstituted with respective solvents petroleum ether, methanol, and water and these were taken in a concentration of 3mg/mL for antimicrobial activity and standard antibiotic-Ofloxacin(10µg) used as positive antimicrobial control, was poured in each well and incubated for 18-24 h. Next day these plates were observed for clear zone around the well if any. The diameter of zones of inhibition (ZOI) was investigated employing a hand-held digital caliper. The data was expressed as mean ±SD. MIC values of the extracts were determined by turbidimetry. The petroleum ether, methanol and water extracts in different concentration were dissolved using DMSO (Dimethyl sulfoxide). Nutrient broth (10mL) was poured in each test tube and subsequently extracts were transferred. Then in each test tube one loop full of stock culture was transferred and incubated at 37˚C for 18-24 h and the lowest concentration of inhibiting microbial growth (no turbidity) was noted down as minimum inhibitory concentration (MIC) by using nephlo-turbidimetry method [17-22].

STATISTICAL ANALYSIS

The data were statistically analysed by using Graph Pad Prism 5 statistical software and results were expressed as Mean ± S.D.

RESULTS AND DISCUSSION

From the result of successive extraction by using different solvents, the percent yield of methanolic, aqueous and petroleum ether extracts was found to be 8.57%, 6.8% and 4.41% respectively. The anti-microbial properties may be associated to the presence of secondary metabolic products of plant. So, the presence of phytoconstituents (Table 1) likes carbohydrates, proteins, alkaloids, flavonoids, tannins and phenolic compounds etc. in various extracts were found to be effective and responsible for antimicrobial action against a wide range of micro-organisms[13,14].

Table 1
Phytochemical tests of various extracts for identification of various phytoconstituents.

Test	I	II	III
Carbohydrates	0	0	+
Protein	0	0	+
Alkaloids	0	+	0
Flavonoid	0	+	0
Tannins and Phenolic compounds	0	+	+

I-Petroleum ether extract, II- Methanol extract, III- Aqueous extract; (0)-Absence, (+)-Presence

The zones of inhibition obtained with petroleum ether, methanol, and aqueous extracts and standard drug are presented in Table 2, Figure 2. It was observed that all the extracts exhibited significant antimicrobial activity against various microorganisms. Among the three solvent extracts, methanolic extract exhibited maximum inhibition against the various organisms compared with other extracts and order of inhibition was found to be *Bacillus subtilis*>*Staphylococcus aureus*>*Saccharomyces cerevisiae* >*Escherichia coli*. In methanolic extract, the maximum zone of Inhibition (ZOI) 24±1.08 mm, in petroleum ether extract the maximum ZOI 16±1.03 mm and in aqueous extract the maximum ZOI 14±0.60 mm was found against *B. subtilis*. In minimum inhibitory concentration (MIC), the methanolic extract exhibited maximum inhibition in comparison to petroleum ether and aqueous extracts. The petroleum ether and aqueous extracts failed to inhibit microbial growth of some organisms as summarized in Table 3 [23,24].

Table 2
Antimicrobial activity (ZOI-Zone of inhibition) of various extracts of leaves of *Euphorbia hirta* on the selected strains of microorganisms.

Test Organism	Diameter of zone of inhibition (mm)#			
	I	II	III	IV
Bacillus subtilis	16±1.03	24±1.08	14±0.60	26±0.60
Escherichia coli	10±0.44	14±0.55	13±0.45	21±1.44
Saccharomyces cerevisiae	13±0.90	15±0.83	12±0.23	17±0.88
Staphylococcus aureus	14±0.80	16±0.30	12±0.78	18±0.95

#N=3±S.D. (Standard deviation);
I- Petroleum ether extract, II- Methanol extract, III- Aqueous extract, IV- Control (Standard drug i.e. Ofloxacin)

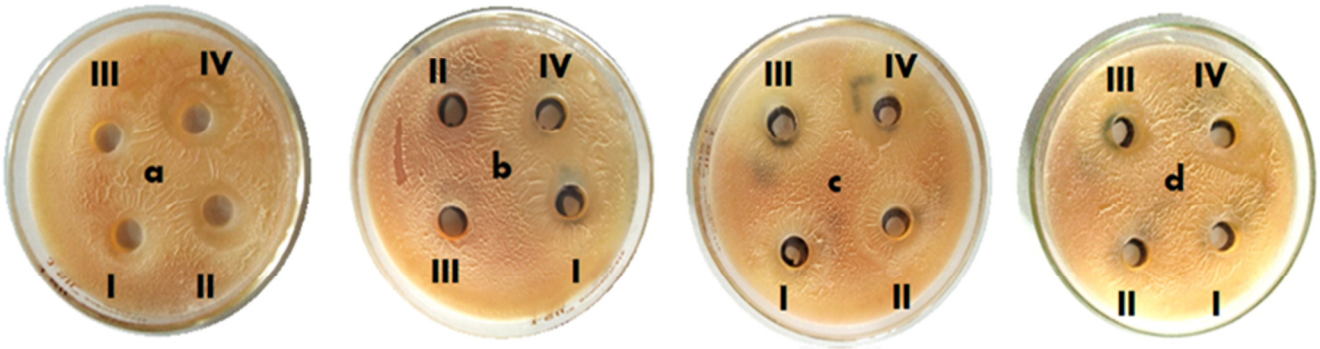

Figure 2
Zone of inhibition (mm) in (a) *E. coli,* (b) *B. subtilis,* (c) *S. cerevisiae and* (d) *S. aureus.*

Table 3
Minimum inhibitory concentration (MIC) of various extracts of leaves of *Euphorbia hirta*.

Test Organism	MICs (µg/mL)		
	I	II	III
Bacillus subtilis	-	220	-
Escherichia coli	-	250	160
Saccharomyces cerevisiae	200	280	-
Staphylococcus aureus	180	220	-

I-Petroleum ether extract, II- Methanol extract, III- Aqueous extract

CONCLUSION

The results concluded that the antimicrobial activity of all the extracts was revealed from moderate to significant across Ofloxacin as a standard but the methanolic extract exhibited significant antimicrobial action in comparison to other extracts due the presence of alkaloids, flavonoids, Tannins and Phenolic compounds. The preliminary phytochemical analysis of various extracts revealed the existence of various phytoconstituents as secondary metabolic products that are potentially responsible for antimicrobial action against a wide range of micro-organisms.

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AUTHOR'S CONTRIBUTION STATEMENT

Dr. Reena Gupta conceptualized and gathered the data with regard to this work. Dr. Jitendra Gupta analyzed these data and necessary inputs were given towards the designing of the manuscript. All authors discussed the methodology and results and contributed to the final manuscript.

CONFLICT OF INTEREST

Conflict of interest declared none.

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