Journal of Phytology 2010, 2(6): 55–60
Pharmacology
ISSN: 2075-6240
Available Online: www.journal-phytology.com

REGULAR ARTICLE

PHARMACOGNOSTICAL AND ANTIBACTERIAL STUDIES OF DIFFERENT EXTRACTS OF *EUPHORBIA HIRTA* L.

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SUMMARY

Leaves of *Euphorbia hirta*, traditionally practiced in the treatments of boils, dysentery, enteritis and various skin conditions, were extracted by soxhlet extraction in various extraction mediums. The disc diffusion method was used to determine the antibacterial activity against many Gram positive and Gram negative bacteria (Standard strains and clinical isolates). Antibacterial sensitivity test indicated that the methanolic extract inhibited the growth of *S. aureus*, *E. coli*, and *B. subtilis* to varying extents while *K. pneumonia* was the most resistive strain to these extracts. Minimum inhibitory concentration (MIC), of the extract against *E. coli*, *S aureus*, and *S. entertidis* were in the range of 0.1mg/ml. Phytochemical analysis indicates presence of terpenes, tannins, alkaloids and flavonoids which might be accountable for its antimicrobial properties, and these results validate the traditional uses of the plant in the treatment of various diseases.

Bhuvaneshwar Upadhyay et al. Pharmacognostical and Antibacterial Studies of Leaf Extracts of *Euphorbia hirta* L. J Phytol 2/6 (2010) 55-60
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1. Introduction

The value of ethnomedicine and traditional pharmacology is these days achieving great appreciation in modern medicine, as the search for new potential medicinal plants is frequently based on an ethnomedicinal basis (Muthu *et al.,* 2006; Parveen *et al.,* 2007; Upadhyay *et al.,* 2010). Ethnobotanical studies of different areas of Rajasthan state has been carried out by many workers in this field (Singh and Pandey, 1998; Mishra and Kumar, 2000, 2001; Katewa *et al.,* 2004; Parveen *et al.,* 2007; Upadhyay et al., 2010).

E. hirta is an Annual plant growing to 0.3m by 0.25m. The plant prefers light (sandy) and medium (loamy) soils and requires well-drained soil. According to survey, different parts of *E. hirta* are used for curing various ailments. The aerial parts of the plant are harvested when in flower during the summer and dried for later use. The stem is used as a treatment for asthma, bronchitis and various other lung complaints. The whole plant is decocted and used in the treatment of athlete's foot, dysentery, enteritis, and skin conditions (Upadhyay *et al.,* 2010). It has been used in the treatment of syphilis. The sap is applied to warts in order to destroy them, and treatment needs to be repeated 2 - 3 times a day over a period of several weeks to be fully effective.

Along with common secondary metabolites like; alkaloid, flavonoids, coumarins and terpenes, a number of substances, as; tannins, gallic acid, quercetin, phenols, phyto-sterols, alcohols, etc. have been reported in the plant (Kerharo and Adam, 1974; Burkill, 1985). Blanc et al., (1972) reported ellagic, Gallic, chlorogenic and caffeic acids, kaempferol, quercitol, quercitrin, and a number of amino acids. The alcoholic extract of the whole plant had an anticancer action in mice (Hartwell, 1967; Sharma and Kumar, 2000). The plant has also been shown to have anti-helminthic activity (Ayensu, 1979; Sofowora, 1993; Adedapo et al., 2005).

Interaction with some traditional medical practitioners revealed that the plant is very popular amongst them, thus there is need to determine its antibacterial potentials. This work was therefore undertaken to

substantiate the antibacterial potentials of *E. hirta.*

2. Material and methods

Extraction of the plants

The leaves of *E.hirta* were collected from many regional areas of Jaipur city, during post monsoon period and were authenticated by botanists at Deptt. of Botany, University of Rajasthan, Jaipur, India and a specimen sample is kept in our institution (herbarium voucher numbers RUBL 20280). Shade dried coarsely powdered leaves (44 g) of *E.hirta* were subjected to successive extraction with various extraction solvents (54-55.5°C) for 24-36 hr using a soxhlet extractor. These crude extracts were concentrated using vacuum evaporator. The dried filtrate was reconstituted with 100% dimethylsulfoxide (DMSO).

Paper disks (diameter 6mm) were then impregnated with 100µl of the final extract, which is equivalent of 10mg/ml of dried plant material. Once the DMSO had evaporated, the disks were placed in a refrigerator and stored in darkness for the duration of the assays. 0.01ml of one of the 24 h broth cultures culture were spread on sterilized nutrient agar media and impregnated discs were placed on it and incubated for 24 h at 37°C.

Preparation of micro-organism culture

In vitro antimicrobial activity of the different extracts of *E. hirta* was studied by disc diffusion method using different concentrations on different microbial strains such as *Escherichia coli* (ATCC 25922 and Clinical isolate), *Proteus vulgaris* (ATCC 13315), *Salmonella enteritidis* (clinical isolate), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538P and clinical isolate), *Pseudomonas aeruginosa* (ATCC 9027 and clinical isolate), *Klebsiella pneumoniae* (ATCC 13883). The bacterial cultures were obtained from, SMS Hospital, Jaipur.

All the bacteria were incubated at 30 ± 0.1°C for 24 hours by inoculation into Nutrient Broth (Sigma). Sterilized Petri dishes (9 cm diameter) were inoculated with 0.01 ml of one of the above culture media (~10^5 bacteria per ml). Muller-Hinton agar (Sigma), sterilized in a flask and cooled to 45–50°C, was distributed by pipette (15 ml) into each inoculated Petri dish and swirled to distribute the medium homogeneously. Discs injected with extracts were applied on the solid agar medium by pressing slightly. The treated Petri dishes were placed at 4°C for 2 hours and then incubated at 35 ± 0.1°C for 24 hours.

At the end of the period, inhibition zones formed on the medium were measured with a transparent ruler in millimeters and compared with the standard drugs prepared by using standard antibiotics as Ampicilin (10µg/ml), Streptomycin (10µg/ml), and Tetracyclin (30µg/ml) in sterile distill water. The experiment was performed in triplicate, and average diameter of zone of inhibition was obtained.

Phytochemical investigation by TLC

The detection of active principles in medicinal plants plays a strategic role in the both; qualitative and quantitative phytochemical investigation of crude plant extracts (Pascual *et al.,* 2002). TLC is a rapid and economical procedure for the determination of the main active principles of medicinal plants e.g., alkaloids, cardiac glycosides, coumarins, flavonoids, saponins, tannins, etc. TLC is also used for fractionation of the extract obtained by extraction procedure by using different solvent compositions.

The extent of the surface of the spot is a measure for the quantity of the material present (Pascual *et al.,* 2002). The volume of the spots applied on the chromatographic plates was 5µl, corresponding to approximately 300µg for each dry extract. Chromatography was performed in the following solvent systems: Nonpolar solvent: toluene-acetone (8:2); semi-polar solvent: toluene-chloroform-acetone (40:25:35); polar solvent: n-butanol-glacial acetic acid-water (50:10:40). The chromatograms were observed first without chemical treatment, under UV 254 nm and UV 365 nm light, and then using the spray reagents.

Determination of Minimum Inhibitory Concentration

For determination of Minimum Inhibitory Concentration (MIC), the method of Cheesbrough (2000) was used. Stock solutions were prepared by dissolving the extracts in DMSO. Two-fold serial dilutions were employed to determine MIC values. Each microorganism was incubated with an extract in duplicate tubes containing a total volume of 10 mL.

The final concentration of extract was in the range 0.1 to 1.5 mg/mL. Control tubes without extract were constituted similarly. Antibiotics were included as positive control in different tubes. The MIC was the lowest concentration of extract with no visible bacterial growth or no turbidity.

3. Result and Discussion

Table 1.Ethnopharmacological studies of *E. hirta*

E. hirta	Whole plant	Amoebic dysentery	Decoction of whole plant is taken internally with milk for 5 days
		athlete's foot	Whole plant is decocted and taken internally and paste is also applied on affected area. in the treatment of athlete's foot
		Skin problems	Crushed whole plant is applied on the affected area in skin conditions
	Leaf	Asthma and bronchitis	The drug is administered in the form of aqueous extract with Grindelia or senega in the treatment
		Leucorrhoea	About 20 leaves are crushed and the extract is given orally with honey once a day in the morning.
		syphilis	Decoction of leaves is taken internally with milk to treat syphilis
	Fresh latex	Warts	The fresh latex is applied to warts and The treatment needs to be repeated 2 - 3 times a day.

Table 2.Antibacterial screening of the different extract of *Euphorbia hirta*

Micro-organisms	*E. hirta* Plant leaf extracts* (10 mg/ml)					Standard Antibiotics**		
	Ace.	Eth.	Aq.	Chl.	Met.	Strepto. 10 µg	Amp. 10µg	Tetra. 30µg
B. subtilis (ATCC 6633)	6.5	11.3	9.4	7.6	12.3	19	15	25
E. coli (ATCC 25922)	12.3	10.2	-	6.9	9.6	19.4	15	-
E. coli (clinical isolate)	12.8	-	11.0	11.3	11.7	20.6	-	27
P. vulgaris (ATCC 13315)	9.3	6.5	8.9	9.4	9.4	18.3	18	25
P. aeruginosa (ATCC 9027)	8.1	7.1	8.9	-	11.2	22.5	-	20
P. aeruginosa (clinical isolate)	8.0	6.5	8.6	-	9.6	20.0	14	12
S. aureus (ATCC 6538P)	13.0	12.0	11.2	11.4	10.6	17.9	15	-
S. aureus (clinical isolate)	12.5	11.6	-	-	13.2	19.5	16	-
S. enteritidis (clinical isolate)	11.3	5.6	10.3	-	8.9	18.6	-	24
K. pneumoniae (ATCC 13883)	-	-	6.5	-	-	20.4	17	26
S.typhae (clinical isolate)	11.0	12.1	9.8	-	8.6	18	19	24

*Ace.=Acetone, Eth.= Ethanol, Aq.=Aqueous extract, Chl.=Chloroform, Met.=Methanol

** Strepto= Streptomycin, Amp= Ampicilin, Tetra= Tetracycline

Table 3.Minimum inhibitory concentrations of the ethanolic extract of *Euphorbia hirta* against test isolates

S.No.	Microorganism	*E. hirta*
1.	*B. subtilis* (ATCC 6633)	0.2
2.	*E. coli* (ATCC 25922)	0.1
3.	*E. coli*(clinical isolate)	0.1
4.	*P. vulgaris* (ATCC 13315)	>0.5
5.	*P. aeruginosa*(ATCC 9027)	NA
6.	*P. aeruginosa*(clinical isolate)	NA
7.	*S. aureus*(ATCC 6538P)	0.1
8.	*S. aureus*(clinical isolate)	0.1
9.	*S. enteritidis*(clinical isolate)	0.1
10.	*K. pneumonia*(ATCC 13883)	1.0
11.	*S.typhae*(clinical isolate)	0.2

Table 4.Preliminary phytochemical screening of ethanolic extract of *Euphorbia hirta*

S. No.	Phytochemical	*E. hirta*
1.	Alkaloid	++
2.	Flavonoids	+
3.	Saponin	-
4.	Coumarins	+
5.	Ployphenols	++
6.	Cardiac glycosides	+
7.	Triterpenes	+++
8.	Cyanogenic glycosides	-

-= (negative result), += (small amount), ++ = (average), +++ = (high), nt= not tested

Plant extracts are generally rich in antimicrobial agents after the flowering (sexual) stage of their growth is complete, and plants taken from stressful environments were particularly active. Antibacterial extracts from plants can be anticipated to be

useful in eliminating infectious diseases. The infecting microorganisms are usually the same as those infecting higher animals and there is therefore compelling reason to suppose that anti-infective agents could be active against human or veterinary pathogens. It is soothing to find, that the spectrum of activity of these plant extracts is broad enough to include human pathogens, as was suggested by folkloric and historical accounts.

During experiment this is noted that leaf extract is more potent than any other extract. Acetone extract was the second more potent extract after methanolic extract. These results are also according to the previous studies of selection of extraction media (Eloff, 1998). As evident by Table 2, the inhibition zone of *S. aureus*by Methanolic extract of leaves of *E. hirta* was 13.2mm, which is highest inhibition zone, received. With observation of results (table 2) it is clear that *E. coli, S. aureus* and *B. subtilis* were the most susceptible bacteria to almost all *E. hirta* extracts. On the contrary, *K. pneumoneae* was the most resistant microorganism, and very less number of the extracts was active against *K. pneumoneae*. Minimum inhibitory concentration of *E.hirta* extracts were also recorded as 0.1 mg/ml in case of *B. cereus, S.aureus*, and *B. subtilis.*

In this study, the results obtained indicated that the Methanolic extract of the E. hirta inhibited the growth of the test isolates except *K. pneumoniae*. This, therefore, shows that the extract contains substance(s) that can inhibit the growth of some microorganisms. Other workers have also shown that extracts of some plants inhibited the growth of various microorganisms at different concentrations (Akujobi *et al.*, 2004; Nweze et al., 2004; Osadebe and Ukwueze, 2004). The observed antibacterial effects on the isolates is believed to be due to the presence of alkaloids, tannins and flavonoids which have been shown to posses antibacterial properties(Cowan, 1999; Draughon, 2004). Some workers have also attributed their observed antimicrobial effects of plant extracts to the presence of the sesecondary metabolites (Nweze *et al.,* 2004) and also identified tannins, flavonoids and alkaloids in the extracts of some medicinal plant (Yoshida et al., 1990; Abo, 1990; Baslas and Agarwal, 1980). The observed antibacterial properties corroborate its use in traditional medicine.

Traditionally, extracts of the plant are used in sore and wound healing, as ear drop for boils in the ear and treatment of boils. They are also used in the control of diarrhea and dysentery. The large zones of inhibition exhibited by the extract against *S. aureus* and *P. aeruginosa* justified their use by traditional medical practitioners in the treatment of sores, bores and open wounds. *S. aureus* and P. aeruginosa have been implicated in cases of boils, sores and wounds (Braude,1982). Also the moderate growth inhibition against *E. coli* justifiesits use in the control of diarrhea and dysentery (Table 1). *E. coli* is the common cause of traveler's diarrhoea and other diarrhea-genic infections in humans. The low MIC exhibited by the extract against *S. aureus* is of great significance in the health care delivery system, since it could be used as an alternative to orthodox antibiotics in the treatment of infections caused by these microbes, especially as they frequently develop resistance to known antibiotics (Singleton, 1999) (Table 3). Their use also will reduce the cost of obtaining health care. The relatively high zone of inhibition exhibited by the extract against *E. coli* is also of significance, since *E. coli* is a common cause of diarrhea in developing countries.

On the basis of the results obtained, it can be concluded that the crude extracts of *E. hirta* exhibit significant antibacterial activity and properties that support folkloric use in the treatment of some diseases as broad-spectrum antimicrobial agents. This probably explains the use of extracts from these species by the indigenous people of South Africa against a number of infections for generations. However, more work needs to be carried out to determine the chemistry of the particular active principle.

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