Evaluation of Antibacterial Activity of a Locally Available Medicinal Plant *Euphorbia hirta*

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Summary: *Euphorbia hirta* L. (Euphorbiaceae) extracted in ethanol (95 %) and subsequent fractionation of ethanol extract into hexane, chloroform and ethyl acetate, exhibited a varied degree of antibacterial activity against wide range of gram positive and gram negative organisms. Alcoholic extract exhibited a more marked and consistent activity against both gram positive and gram negative organisms. *Euphorbia hirta* extract was found to be more active against gram positive organisms as compared to gram negative organisms. Ethanol extract of *Euphorbia hirta* was found to be highly active against troubleshooting gram positive organism *Bacillus subtilis* and gram negative *Pseudomonas aeroginosa* where reference standard fails.

Introduction

Euphorbia hirta synonym *Euphorbia pilulifera* belongs to the family Euphorbiaceae [1-5]. The family is represented by 300 genera and 7500 species, which are well known for their important economic uses as foodstuff and medicine. *Euphorbia hirta* plant is a pantropic weed, widely distributed in tropical countries including Pakistan, India and Northern Australia found abundantly on waste places and grasslands. It is known by varied names such as dudhi, pill bearing spurge, snakeweed, asthma weed etc. [1, 3, 6, 7]

A conclusive data is available on the therapeutic potential of the plant which reveals that the plant possess antiamoebic, antidysenteric, antispasmodic [8-10] anti-inflammatory, analgesic, sedative, diuretic [1,11-14] anti-asthmatic [1, 2, 5, 6] antiseptic, anti-cancer and antileukemic [1, 5, 6] activity.

Literature survey reveals that, beside terpenoidal constituents, a variety of other natural products have so far been reported from this species [5, 15].

Results and Discussion

The present activity is under taken to evaluate the antibacterial activity of crude extract and various fractions of *E. hirta*. This is a part of our ongoing studies on discovery of plant based antibacterial formulations [16]. Ethanol extract and its fractions in hexane, chloroform and ethyl acetate exhibited antibacterial activity against wide range of gram positive and gram negative organisms (Table-1 and 2). DMF was taken as control which exhibited antibacterial activity against some gram positive and gram negative organisms' upto 14 mm size of zone of inhibition. Hence a zone of inhibition of 14 mm or less is considered as no activity.

Ethanol extract showed maximum antibacterial activity against all microorganisms tested while its fraction in chloroform and ethyl acetate showed moderate activity and hexane fraction showed minimum activity.

Ethanol extract showed mild antibacterial activity at 1 % concentration against all microorganisms tested, except *Bacillus purmilus, Salmonella typhi* and *Salmonella typhi* para A which showed resistance at this concentration. Whereas 2 % concentration of extract exhibited moderate antibacterial activity against all the microorganisms except *Salmonella typhi* which showed resistance at all the concentration. The 5 % concentration of ethanol extract exhibited significant antibacterial activity against all the gram positive and gram negative bacteria.

Antibacterial activity of all fractions at different concentrations is compared with standard reference antibiotic ampicillin. According to results summarized in Table-1 and 2, hexane and chloroform

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Table – 1: Antibacterial activity exhibited by *Euphorbia hirta* L. against Gram Positive Organisms

Sr. No.	Name of microorganisms	Zone of inhibition measured in mm															
		Etahanolic Ext.			Hexane Ext.			Chloroform Ext.			Ethyl acetate Ext.			Ampicillin Standard			Negative Control
		Concentration of Extract used (Percentage)															
		0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0	DMF
1.	Bacillus subtilus	D	A	A1	-ve	C	B	D	B	A	C	B	A1	-	-	-	
2.	Bacillus cereus	C	A	A1	D	C	B	D	C	B	D	C	B	D	B	B	
3.	Bacillus pumilus	-ve	D	B	-ve	C	B	C	B	A	C	B	A	D	C	B	
4.	Staphylococcus aureus	B	A	A1	D	B	A	D	B	A	D	C	B	D	C	B	
5.	Staphylococcus epidermidas	C	B	A1	C	B	B	-ve	D	C	-ve	D	C	D	C	B	
6	Streptococcus faecalis	D	D	C	D	D	C	D	B	A	D	C	B	-	-	-	
7.	Streptococcus intermedius	C	B	A1	D	C	A	D	C	B	-ve	-ve	-ve	D	B	A	
	Percent Activity	100%			100%			100%			85-71%			71-42%			

Categorization of Zone Inhibition:
15 – 19 mm D
20 – 24 mm C
25 – 29 mm B
30 – 34 mm A
35 – 39 mm A1
09 – 14 mm Negative

Table – 2: Antibacterial activity exhibited by *Euphorbia hirta* L. against Gram Negative Organisms

Sr. No.	Name of microorganisms	*Zone of inhibition measured in mm*															
		Etahanolic Ext			Hexane Ext.			Chloroform Ext.			Ethyl acetate Ext.			Ampicillin Standard			Negative Control
		Concentration of Extract used (Percentage)															
		0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0	DMF
1.	Escherichia coli	D	B	A	D	C	B	D	C	A	D	C	B	D	B	A	
2.	Klebsiella pneumoniae	C	A	A1	-ve	-ve	-ve	-ve	D	C	D	B	A	D	C	B	
3.	Salmonella typhi	-ve	-ve	-ve	D	C	B	-ve	-ve	-ve	-ve	-ve	-ve	D	C	B	
4	Salmonella typhi para A	-ve	D	C	D	C	C	-ve	D	C	D	C	B	-ve	-ve	-ve	
5	Salmonella typhi para B	D	C	B	D	C	B	D	B	A	D	B	A	D	C	A	
6.	Proteus mirabilus	D	C	B	D	C	B	D	B	A	D	B	A	-ve	-ve	-ve	
7	Pseudomonas aeroginosa	B	A	A1	-ve	-ve	-ve	-ve	-ve	-ve	D	C	B	-ve	-ve	-ve	
8	Shigella dysenteriae	B	A	A	D	C	B	D	C	B	D	C	B	D	C	B	
9.	Shigella flexaneriae	C	B	B	D	D	C	D	C	B	D	D	C	D	C	B	
10	Yersinia aldovae	C	B	A1	D	C	B	D	B	A	D	B	A	D	C	C	
	Percent Activity	90%			80%			80%			90%			70%			

Categorization of Zone Inhibition:
15 – 19 mm D
20 – 24 mm C
25 – 29 mm B
30 – 34 mm A
35 – 39 mm A1

fractions exhibited pronounced activity i.e. 100 % against gram positive organisms and 80 % against gram negative organisms, hence activity increases with increase in concentration of extracts. While ethyl acetate fraction exhibited 85.71 % activity against gram positive and 90 % against gram negative organisms.

In general the antibacterial activity exhibited by *E. hirta* extract and fractions in different solvents against gram positive and gram negative organisms is much better as compared to reference standard. It was interesting to note that the plant extract was found to be highly active against *Bacillus subtilis* and *Pseudomonas aeroginosa*, which in most cases are resistant to most antimicrobial agents. Furthermore *E. hirta* plant extracts were found to be more active against gram positive group organisms as compared to gram negative organisms. The difference in activity observed between test and reference standard clearly indicates that the indiscriminate use of antibiotics has developed resistance against microorganisms. Hence it is concluded that plant based antimicrobials can play an effective role in the treatment of infectious diseases and also eradicate number of side effects i.e. hypersensitivity, immune suppression and allergic reactions related to synthetic antimicrobials [16]. Ethanolic extract is more active as compare to its fractions because of the combined effect of biologically active secondary products i.e. flavonoids, glycosides, alkaloids, tannins, steroids, plant acids and phenolic compounds. These compounds are more complex and specific in nature and *in vitro* have been found to exhibit antibacterial activity [17].

Study fully validate that the locally available medicinal plant *E. hirta* L. is as efficient in activity and potentiality as the plant found in other part of the world.

Experimental

Material and Method

The plant material was collected from Karachi and identified by a taxonomist and a voucher

specimen was deposited in the herbarium of Department of Botany, University of Karachi (Pakistan).

Preparation of Extract

The collected plant material was washed thoroughly dried in shade and milled. The dried and milled plant (5 kg) was soaked in 10 L of 95 % ethanol for 96 hours at room temperature and then decanted and pooled in a well stoppered container. Process was repeated thrice in order to have maximum quantity of extract. Pooled solvent was removed under vacuum at 60 ^{0}C ± 1 ^{0}C. This yielded a crude ethanolic extract (2.4 %). Ethanolic extract was successively extracted exhaustively with petroleum ether (40-60 ^{0}C), chloroform and ethyl acetate. Percentage yield of each solvent extract was 15 % n-hexnane, 25 % chloroform and 18% ethyl acetate. All fractions were kept in well stoppered glass containers for further study.

Preparation of Solution

All extracts (i.e. ethyl alcohol, pet ether, chloroform and ethyl acetate) were dissolved respectively in dimethyl formide (DMF) to give a strength of 20 mg/ ml (i,e. 2 %) from which further dilution of 0.5 and 1.0 % were made. Each cavity/well received 0.25 ml solution of their respective concentration. Ampicillin was used as a reference standard in same strength/concentration.

Microorganism Used

The said activity were assessed against a wide range of gram positive and gram negative organisms. The microorganisms were procured from the department of Microbiology, University of Karachi. Before assessing the antibacterial activity purity of the cultures was fully ascertained by standard methods of characterization.

Culture Media and Inocula preparation

All the test organisms were maintained on tryptic soya agar slants at 4 ^{0}C prior to testing.

The test culture inocula was prepared by using tryptic soya broth culture medium maintained at 37 ^{0}C ± 1 ^{0}C for 22 hours. For the antibacterial assay standardized inocula containing 10^6 CFU/ ml was used.

Antibacterial Activity

The antibacterial activity was carried out by Agar plate diffusion method (Perez C *et al* 1990) using 10^6 CFU/ ml of the inocullum. The inoculum (1 ml) was thoroughly mixed with 25 ml of sterile melted soya agar maintained at 40-45 ^{0}C and poured into presterilized petdishes respectively and marked accordingly. All plates were left to set at 4 ^{0}C for 15-30 minutes and holes of 9 mm were made in the centre of each seeded plate. Holes were than filled aseptically with 0.25 ml of prepared solutions (i.e. test, standard and solvents only negative control) respectively and marked accordingly. All plates were then incubated at 37 ^{0}C ± 1 ^{0}C for 22 hours and zone of inhibition exhibited were measured and recorded accordingly. Each concentration of each solvent was run in triplicate and whole set was repeated thrice to confirm the results.

References

1. J. M. Watt and B. B. WijkA, *The Medicinal and Poisonous Plants of Southern Eastern Africa.* 2nd edition Livingstone, Edinburgh, London, 1251 (1962).
2. L. M. Perry and J. Metzger, *Medicinal Plants of East and South East Asia* MIT Press Cambridge, London, 1 (1980).
3. R. N. Chopra, S. L. Nayar and I. C. Chopra, *Glossary of Indian Medicinal Plants* CSIR, New Delhi, India 1273 (1986).
4. A. Chavallier, *The encyclopedia of Medicinal Plants* Darlings Kindersley, London (ISBN-9-780751-303148) (1996).
5. S. S. Bhatnagar, *The Wealth of India*, CSIR, New Delhi, India, 225 (2003).
6. S. H. Abdallah, *Euphorbia Species,* Lancet, **15**, 339 (8790): 434 (1992).
7. H. M. Burkill, *The useful Plants of West Tropical Africa,* Roy Bot. Gard Ken, 2, 12-150 (1994).
8. F. M. Paul, G. Kamini, F. Mclanic, F. Charles and B. Bilong, *Afr. J. Trad. Cam.* **2**: 113 (2005).
9. S. K. Hore, V. Ahuja, G. Mehta, P. Kumar, S. K. Pandy and A. H. Ahmed, *Fitoterapia*, **23**, 125 (2005).
10. M. C. Lenher, J. Fleurentin, P. Dorfman, F. Mortier and J. M. Pelt, *Planta Medica,* **57**(3), 225 (1991).

11. P. B. Johnson, E. M. Adurahman, E. A. Tiam, I. Abdu Aguye and I. M. Hussaini. *J. Ethanopharmacol,* **65**(1), 63 (1999).
12. K. Vijaya, S. Ananthan and R. Nalini, *J. Ethanopharmacol,* **49**(2) 115 (1995).
13. E. Thomas, J. Shanmugan and M. M. Rafi, *Biomedicine,* **19**(3), 185 (1999).
14. J. Parekh, D. Jadeja and S. Chandra, *Turk J. Biol.,* **29**, 203 (2005).
15. J. Hohman, R. Molnar, D. Redei, F. Evanics, P. Forgo, A. Kalman, J. Argey and P. Szabo, *J. Med. Chem.,* 6, **45**(12), 2425 (2002).
16. S. Ahmed, K. Fatima and Atiq-ur-Rahman, *Pak. J. Sci. Ind. Res.,* **47**(1), 29 (2004)
17. H. Hamburger and Hostetmamn, *Phytochemistry,* **30**: 3864 (1991).