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ANTIBACTERIAL ACTIVITY OF *EUPHORBIA HIRTA* EXTRACTS

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ABSTRACT
Deadly diseases of this century were mainly caused by bacterial and fungal infections. Biologically important plants were discovered by evaluation of ethno pharmacological data and they are locally populated with immediate therapeutic action. Because of severe toxic effects produced by today's synthetic drugs the alternative medical system has come into existence. These alternative systems were devoid of the side effects produced in the body up to allopathic drugs were concerned. The aim of the current study was to investigate antibacterial activity of the ethanol and petroleum ether extracts of *Euphorbia hirta* L against clinically important bacterial sp. Different concentrations of crude drugs such 25µg/ml, 50µg/ml, 75µg/ml, and 100µg/ml were prepared and antibacterial activity was found by using cup-plate method. The result from this study thus showed that ethanol extracts of *Euphorbia hirta* have potentially deleterious effects on micro-organisms.
Keywords: Antibacterial activity, Cup-plate method, *Euphorbia hirta*, Ethanol, Pet ether, soxhelet extractor

INTRODUCTION

Today's world's mortality rate depends on the infectious diseases and accounts for about 50% of all deaths. In Bangladesh about 17% of children were died due to diarrhea[1]. Some 5.8 million deaths of infants and children were caused by enteric diseases worldwide[2]. Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbours an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components.

This study is aimed at exploring the antibacterial activity of the plant extract and comparing this with standard antibiotic like tetracycline and kanamycin. *Euphorbia hirta* is a small annual herb common to tropical countries[4]. It can grow to a height of 40 cm. The stem is slender and often reddish in colour, covered with yellowish bristly hairs especially in the younger parts. The leaves are oppositely arranged, lanceolate and are usually greenish or reddish underneath measuring about 5 cm long. In the axils appear very small dense round clusters of flowers. The small green flowers constitute the inflorescence characteristic of the euphorbia's. The stem and leaves produce white or milky juice when cut[5,6].

MATERIALS AND METHODS

Collection of plant material

The fresh plant of *Euphorbia hirta* was collected from campus of QIS College of Pharmacy, Vengamukalapalem, Ongole, Prakasam district, Andhra Pradesh state, India. *Euphorbia hirta* belongs to the family Euphorbiaceae.

Figure 1: Different plant parts of *Euphorbia hirta*

Preparation of the extracts

After collection of plants, they were washed by running tap water and every part of the plant was separated into roots, stem, bud, leaves and shadow dried for 25-30 days. After proper drying, they were made to powder separately. 100g of each powder sample was taken and soaked in 500 ml of solvent (55˚C- 60˚C) in soxhelet extractor and kept for extraction about 8 cycles in 15 to 18 hrs[7]. The solvents used were ethanol and petroleum ether due to their non-polar nature.

After extraction of powder sample, the crude extract was collected, evaporated to dryness, extracts were concentrated using vacuum evaporator and stored properly in moisture free container. The methods[8] were adopted for the preparations of dilutions of crude extract for antibacterial assay. The extracts were dissolved as per mg/ml in same solvents and further dilutions were made as 25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml.

Preparation of standard bacterial suspensions

The minimum number of viable organisms of *Staphylococcus aurous, Salmonella typhi, Pseudomonas aureginosa, Vibrio cholera, Escherichia coli* per ml of stock suspension was found by using surface viable counting technique of colony counter. About 10^8-10^9

colonies can be identified. By maintaining constant experimental conditions, each time a fresh stock suspension can be prepared.

Antibacterial Activity

The antibacterial activity of *Euphorbia hirta* was found by using cup plate method. It is one of the easiest and economic methods of finding antibacterial activity. The cup plate method as described by Saravanan *et al* was adopted for the study[9]. About 15 ml of sterile Muller Hinton agar (Himedia) in a Petri dish was seeded with 1.0 ml of standardized broth cultures of the bacteria (1.0 x 107 cfu/ml) and spread gently to ensure uniform distribution of the microorganisms and then allowed to solidify on a flat surface. Four wells were made in the plates (about 5.0 mm diameter) using a sterile cork borer and 25µg\ml, 50µg\ml, 75µg\ml, 100µg\ml of the extracts were transferred into the well using a micro pipette. The plates were allowed to stand for one hour for pre-diffusion of the extract to occur[7] and were incubated at 37 ^{0}C for 24 hours. After incubation for 24 hours, the zone of inhibition was calculated by measuring the diameter of zones of growth inhibition by using colony counter[10].

Table 1: Micro-organisms and their maintenance

Micro organism	MTCC number	Temperature required for growth of micro organism (°C)	Incubation Time (Hours)
Pseudomonas aureginosa	1954	37	48
Streptococcus aurous	3160	37	24
Salmonella typhi	733	37	12
Vibrio cholera	3904	37	12
Escherichia coli	1652	37	24

Table 2: The antibacterial activity of *Euphorbia hirta* by cup plate method

Plant part	Solvents	Concentration (µg/ml)	Zone of inhibition (mm)				
			S. aureous	*P.aureginosa*	*S.typhi*	*E.coli*	*V.Choler*
leaf	Ethanol	25	18.4	19.5	19.1	21.2	16.5
		50	18.2	17.8	20.4	26.4	16.1
		75	20.5	17.2	19.7	28.	14.4
		100	19.1	20.1	18.4	26.2	19.7
	Petroleum ether	25	10.0	9.3	9.1	7.1	8.1
		50	10.4	10.4	9.5	8.3	8.6
		75	10.5	10.6	10.2	6.5	8.4
		100	8.3	9.2	10.7	8.4	8.8
Stem	Ethanol	25	13.6	13.3	12.8	13.3	16.6
		50	13.5	14.0	16.2	19.1	14.1
		75	16.7	22.2	33	15.2	13.2
		100	18.4	18.9	24.7	13.5	13.4
	Petroleum ether	25	7.2	8.3	10.9	7.4	17.8
		50	7.2	8.3	11.8	8.6	16.6
		75	6.5	8.0	11.6	7.8	6.4
		100	7.7	7.2	12.2	7.5	7.5
Root	Ethanol	25	15.8	16.1	18.0	7.2	15.2
		50	14.8	18.3	18.2	7.1	11.0
		75	20.1	14.5	15.2	8.3	13.8
		100	16.2	22.4	24.5	2.5	21.9
	Petroleum ether	25	12.3	6.6	7.5	12.9	6.6
		50	8.7	11.8	7.6	14.7	8.0
		75	8.4	15.9	8.3	17.2	11.3
		100	8.8	16.2	8.2	11.6	15.3
Bud	Ethanol	25	18.9	19.3	17.1	21.2	9.1
		50	17.1	17.7	18.4	21.7	17.2
		75	18.2	18.2	20.5	23.3	21.6
		100	19.6	19.5	25.3	24.9	19.4
	Petroleum ether	25	12.5	15.3	10.1	10.2	11.6
		50	12.5	12.5	14.6	10.8	11.8
		75	13.5	10.7	14.5	12.2	11.2
		100	14.0	10.2	13.2	27.7	13.1

RESULTS AND DISCUSSION

Results of the antibacterial screening of the different concentrations of the extract on the test isolates are shown in Table 2. The results show that increase in concentration of extract increased the zone of growth inhibition of some of the microorganisms. From this study, it was concluded that ethanol extract of *Euphorbia hirta* stem was active against *Salmonella typhi.* This therefore shows that it is used in treatment of typhoid and other large zones produced by *Escherichia coli* and controls diarrhoea and dysentery. *Escherichia coli* was common cause for Traveller's disease and other diarrhoeagenic infections in humans[11]. The low MIC produced against *Staphylococcus aurous* is of high importance in health care delivery systems because it is used as an alternative to orthodox antibiotics in these infections[12]. *Staphylococcus aurous* releases the enzyme penicillinase that converts antibiotic penicillin into penicillinoic acid which is longer inhibitory to its growth[13]. Preliminary phytochemical screening of ethanolic extract of *Euphorbia hirta* shows the presence of Plant Tannins, Flavonoids , Alkaloids , Cardiac glycosides and absence of Saponins, Cyanogenic glycosides.

Thus *Euphorbia hirta* was found to contain many bioactive components with antibacterial activities. Further Pharmacological studies is needed to separate active constituents and evaluate their anti bacterial activity towards broad range of microbial pathogens.

CONCLUSION

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[14]. They are also used in the control of diarrhoea and dysentery[15, 16]. From the obtained result it was showed that this type of antibacterial evaluation has provided accurate zone of inhibition of various micro organisms[2]. Thus the constituents of *Euphorbia hirta* can be used as antibacterial purpose[17]. It can also be used as diuretic and purgative action. It is also a well known remedy for inflammation of respiratory tract and for asthma as it has a special reputation for causing bronchial relaxation. The stem is used to treat asthma, bronchitis and lung complaints[18]. The whole plant has a wide use in Athlete's foot, dysentery and enteritis and skin conditions and also syphilis[19].The roots is used to treat warts to destroy them with repeated treatment of two times a day. It contains secondary metabolites like alkaloids, flavonoids, coumarins and terpenes and tiny portions of tannins, Gallic acid, phenols, phyto- sterols, alcohols, amino acids, caffeic acid etc[20]. Therefore, our results revealed the importance of plant extracts when associated with antibiotics, to control resistant bacteria, which are becoming a threat to human health. Furthermore, in a few cases, these plant extracts were active against antibiotic resistant bacteria under very low concentration, thus minimizing the possible toxic effects.

REFERENCES

1. Alam NH, Faruque AS, Dewan N, Sarker SA, Fuchs GJ. Characteristics of children hospitalized with severe dehydration and persistent diarrhea in Bangladesh. J. Pharm Pharmacol 2001; 53:18-24.
2. Amita S, Chowdhoury R, Thungaatia M, Rommuthy T, Nair GB, Ghosh A Class A Integrons and SXT Elements in EL Tor strains isolated before and after 1992 Vibrio Cholera 0139 outbreaks, Calcutta, India, Emerg, Infect 2003; Dis 9(4): 112-128.
3. Soforowa EA. Medicinal plants and traditional medicine in Africa. John Wiley and Sons, Chichester; 1982: 198.
4. Lind EM, Tallantire AC. Some Common Flowering Plants of Uganda. Oxford University Press: Nairobi; 1971: 182.
5. Anononymous. The use of *Euphorbia hirta* in the treatment of sores boils and wounds. Personal communication with the Chairman, Imo state Traditional Medicine Practioners: Owerri Nigeri; 2005.
6. Esimone CO, Adiukwu MU, Okonta JM. Preliminary Antimicrobial Screening of the Ethanolic Extract from the *Lichen Usnea subfloridans* (L). J. Pharmaceutic. Res. Dev 1998; 3(2): 99 – 102.
7. Saravanan R, Dhachinamoorthi D, Sanjith Nath M., Senthilkumar K. Antimicrobial Properties of Passiflora foetida L. against Various Clinical and Phytopathogens. Inventi Rapid: Ethnopharmacology 2010; 1(2): 1-3.
8. Saravanan. R, Dhachinamoorthi. D, Senthilkumar. K and Thamizhvanan. K. Antimicrobial activity of various extracts from various parts of *Calophyllum inophyllum* L. Journal of Applied Pharmaceutical Science 2011; 01 (03): 102-106.
9. Ogbolie JN, Ogeke CC, Okoli IC, Anyanwu BN. Antibacterial activities and toxicological potentials of crude ethanolic extracts of *Euphorbia hirta* Afr.J. Biotechnol 2007; 6(13):1
10. Doughari JH, EI-Mahmood AM, TyoyinaI. Antibacterial activity of leaf extracts of Senna obtusifolia. Afr.J.Pharm.Pharmacol 2008; 2 (1):007-013.
11. Lino A, Deogracious O. The in-vitro antibacterial activity of Annona senegalensis, Securidacca, Longipena, Steganotaenia araliicea-Ugandan Medicinal Plants. Afr.Health Sci 2006; 6(1):31-35.
12. Darwish RM, Aurjai I, AI-Khalil N, Mahafzah A (2002). Screening of antibiotic resistant inhibitors from local plant material against two strains of Staphylococcus aurous .J.E thnopharmacol.76:359.
13. Singleton, P. Bacteria in Biology, Biotechnology and Medicine. 1548 4th edn. John Wiley and Sons Ltd; New York: 1999.
14. Akujobi, C., Anyanwu, B.N., Onyeze, C., Ibekwe, V.I. Antibacterial Activities and Preliminary Phyto-chemical Screening of Four Medicinal Plants .J. Appl. Sci 2004; 7(3): 4328.
15. Kokwaro JO. Medicinal Plants in East Africa. 2nd edn. East African Literature Bureau, Nairobi; Kenya: 1993
16. Akinpelu DA, Onakoya TM. Antimicrobial activities of medicinal plants used in folklore remedies in south-western Nigeria, Afr.J. Biotechnol 2006; 5 (11):1078-1081.
17. Mordi RM, Erah PO. Susceptibility of common urinary tract isolates to the commonly used antibiotics in a tertiary hospital in southern Nigeria. Afr.J.Biotechnol 2006; 5 (11):1067-1071.
18. Muhammad HS, Muhammad S. The use of *Lawsonia inermis* Linn.(henna)in the management of burn wounds infections. Afr.J.Biotechnol 2005; 4(9):934-937.
19. Kambezi L. Afolayan,AJ. Extracts from Aloe ferox and *Withania somnifera* inhibit *Candida albicans* and *Neisseria gonorrhoea*.Afr.J.Biotechnol 2008; 7(1):012-015.
20. Adesokan A A, AkanjiMA, Yakuba MT. Antibacterial potentials of aqueous extract of *Enantia chlorantha* stem bark. Afr.J. Biotechnol 2007; 6 (22):2502-2505.

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