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Antibacterial activity of *Euphorbia antiquorum* Linn. plant latex

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Abstract

Euphorbia antiquorum L. Euphorbiaceae is a potent medicinal plant in the traditional Indian medicinal system. Traditionally the latex of *Euphorbia antiquorum* is used in the treatment of ear ache, ulcers, asthma, stomach distension, neuropathy and skin diseases. In the present study the disc diffusion method was used to determine the antibacterial activity of *Euphorbia antiquorum* latex extract against *Escherichia coli*, *Staphylococcus aureus, Proteus vulgaris*, *Bacillus subtilis*, *Micrococcus luteus* and *Vibrio parahaemolyticus*. The antibacterial sensitivity test indicated that the methanolic extract inhibited the growth of the bacterial isolates at varying extents.

Keywords

Antibacterial activity, Disc diffusion method, latex, *Euphorbia antiquorum* L.

Introduction

Infectious diseases are a leading cause of death world wide and antibiotic resistant has become a global concern (Westh *et al*., 2004). One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents (Shah, 2005). These problems highlight the urgent need for new strategies and new class of antibiotics (Adcock, 2002; Rosato *et al*., 2007). About 80% of individuals from certain countries still use plants as remedies for many diseases, using their own personal recipies which have been passed through generations (WHO, 2005).

Plants are the biosynthetic laboratory of chemicals. The secondary metabolites found in the plant latex such as essential oil, tannins, terpenoids, alkaloids and plant extract are known to possess, insecticidal, antibacterial and acaricidal activity (Tepe *et al*., 2004). Therefore they have been intensively screened and applied in pharmacology, pharmaceutical botany, medicinal and clinical laboratory for their therapeutic properties, safety and efficiency (Daferera *et al*., 2000 and Eloff, 1998).

Euphorbia antiquorum Linn. (Euphorbiaceae) is a plant commonly distributed throughout the tropics of Asia, Africa and Middle East Asia. The plant is popularly known due to the abundance of green latex in its green parts which is easily collected when the plant is wounded such a fact reinforces the idea that the milky latex is accumulated as a defense strategy against bacteria, fungi and insects (Deepak, 1995). Several reports indicate many therapeutic activities of *Euphorbia antiquorum* latex, including analgesic, antimicrobial, antimalarial insecticidal and bactericidal activities (Cateni *et al*., 2003)

This paper explores the antibacterial activities of different solvent extract of *Euphorbia antiquorum* latex against common human pathogens.

Materials and methods

Preparation of plant latex:

Fresh latex was collected from the plant early in the morning and was stored in a sterile plastic container. 1ml of the latex was mixed with 9ml of different solvents like methanol, ethanol, and chloroform and petroleum ether and was centrifuged at 3500 rpm for 5 minutes and the supernatant was collected. The collected crude extract was used for preparing discs for testing antibacterial activity.

Disc Diffusion Method

The antibacterial assay was performed by adopting disc diffusion method (Singh *et al*., 2004). The disc carrying the latex extracts were tested against the Gram positive bacteria such as *Staphylococcus aureus, Proteus vulgaris* and *Bacillus subtilis* and Gram negative bacteria such as *Escherichia coli, Micrococcus luteus* and *Vibrio parahaemolyticus*. The sterile filter paper discs were soaked with 100µl of different latex extracts and were dried for 30 minutes. The prepared agar plates were seeded with each of the test bacteria and the filter paper discs soaked with different solvent extracts of the latex were arranged at regular intervals inside the petriplates. The standard reference antibiotic disc kanomycin was placed at the centre of the plate. The plates were incubated overnight at 37° in an incubator. Three replicates were maintained for each extract.

The diameter of the zone of inhibition was measured and recorded in millimeters.

Results and Discussion

Plant extracts are generally rich in antimicrobial compounds. Infection causing bacteria are rapidly becoming resistant to conventional drugs for example methicilin and vancomycin resistant *Staphylococcus aureus* (Machado *et al*., 2008). Scientists are now working to explore alternative drugs from plant sources to explore new and potent antibacterial principles. In the continuation of new antibacterial drug discovery various solvent extracts of latex of *Euphorbia antiquorum* were investigated. Further data obtained showed that the antibacterial activity of plant parts depended largely upon the extraction procedure, type of solvent used for extraction and the bacterial strains used.

In vitro antibacterial activity of the latex *Euphorbia antiquorum* treated with methanol, petroleum ether, ethanol and chloroform extracts with kanomycin as the standard are shown in the Table. The methanolic extract of the *Euphorbia antiquorum* latex had the greatest antibacterial efficacy followed by ethanolic extract and chloroform extract. Petroleum ether extract did not inhibit the growth of the micro organisms used.

The highest zone of growth inhibition of 16mm diameter was exhibited by methanolic extract of latex against *Vibrio parahaemolyticus,* only ethanolic and methanolic extract of latex showed highest inhibition against the tested microorganisms, while the chloroform extract of latex had no effect against *Bacillus subtilis* and *Staphylococcus aureus.* The lowest zone of growth inhibition was observed with chloroform extract of latex against *Vibrio parahaemolyticus* which gave a zone of inhibition measuring 5mm.

One way ANOVA revealed that *Euphorbia antiquorum* latex showed a significant resistance ($F=17.60534$; $df=29$; $P < 0.05$) against the selected bacterial strains.

From the results it was found out that the methanolic extract of latex was more potent than any other extract. Ethanolic extract was the second potent extract after methonolic extract. The results were in accordance with the work of Upadhay *et al*. (2010) who reported that the methanolic extract of *Euphorbia hirta* showed a highest zone of inhibition against the tested bacterial strains. The observed antibacterial effects on the isolates were believed to be due to the presence of alkaloids, tannins and flavonoids which have been shown to possess antibacterial properties (Cowan, 1999). Some workers have also attributed their observed antimicrobial effect of plant extracts to the presence of secondary metabolites (Nweze *et al*., 2004) and also identified tannins, flavonoids and alkaloids in the extracts of plants.

Traditionally extracts of the plant are used in sore and wound healing as ear drop for boils in the ear and treatment of boils. The larger zones of inhibition exhibited by the extract against *Staphylococcus aureus* justified their use by traditional medicinal practitioners in the treatment of boils, sores and wounds (Braude, 1982). 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 diarrhoea in developing countries.

On the basis of the results obtained, it can be concluded that the crude latex extract of *Euphorbia antiquorum* exhibited significant antibacterial activity and properties that support folkloric use in treatment of some diseases.

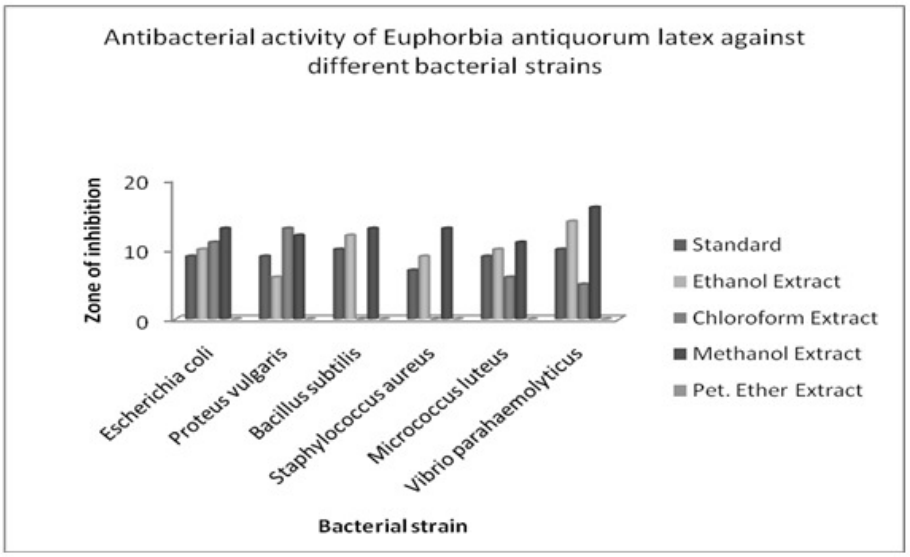
Conclusion

In conclusion among the four solvents used for extraction, methanolic extract of *Euphorbia antiquorum* latex was found to possess best antibacterial activity followed by ethanol and chloroform extracts. This may be due to the high solubility of active compounds of latex.

Antibacterial activity of *Euphorbia antiquorum* Linn. latex extracts against Gram positive and Gram negative bacterial strains

Name of the bacteria tested	Zone of inhibition (mm)				
	Standard	Ethanol Extract	Chloroform Extract	Methanol Extract	Pet. Ether Extract
Escherichia coli	9±0.8164	10±0.8164	11±0.8164	13±0.8164	NA
Proteus vulgaris	9±0.8164	6±0.8164	13±0.8164	12±0.8164	NA
Bacillus subtilis	10±0.8164	12±0.8164	NA	13±0.8164	NA
Staphylococcus aureus	7±0.8164	9±0.8164	NA	13±0.8164	NA
Micrococcus luteus	9±0.8164	10±0.8164	6±0.8164	11±0.8164	NA
Vibrio parahaemolyticus	10±0.8164	14±0.8164	5±0.8164	16±0.8164	NA

Mean value of three replicates



One way ANOVA comparison of antibacterial activity of *Euphorbia antiquorum* latex extract against different bacterial strains

Groups	*Count*	*Sum*	*Average*	*Variance*
Standard	6	54	9	1.2
Ethanol Extract	6	61	10.16667	7.366667
Chloroform Extract	6	35	5.833333	29.36667
Methanol Extract	6	78	13	2.8
Pet. Ether Extract	6	0	0	0

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	591.5333	4	147.8833	18.15262	4.15E-07	2.75871
Within Groups	203.6667	25	8.146667			
Total	795.2	29				

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