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Antibacterial activity of *Euphorbia antiquorum* latex

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Abstract:

Euphorbia antiquorum is a large shrub or small tree belonging to the largest and the most diverse family in the plant kingdom, Euphorbiaceae. *E. antiquorum* latex was used as a classical binding agent for the kshara sutra in the management of the anal fistula.

In the present study, an attempt was made to evaluate the antimicrobial properties of latex of *E. antiquorum* on certain microbes. The methanolic extract of the latex was prepared. The fractions of methanol extract were tested for their antibacterial activity against the Gram positive bacteria, *Streptococcus agalactiae* and *Staphylococcus aureus* and Gram negative bacteria *Escherichia coli* and *P. aeruginosa*.

The results revealed that the latex showed minimum inhibition only to *E. coli* and *S. aureus*. Latex did not possess antibacterial activity against *S. agalactiae* and *P. aeruginosa*. The results suggest that the *E. antiquorum* latex extract account for the antibacterial properties and has a potential for use as an antimicrobial agent.

1. Introduction

In recent years, considerable interest has been evidenced by the medical professionals regarding the use of indigenous drugs in the treatment of diseases (Ananth et al., 2010). The toxic effects produced by the administration of drugs are much more a serious problem than that of the disease itself. These factors compel us to search for safe formulation from alternative medicinal system, which is devoid of side effects in the body (Siddique et al., 2010). Herbal drugs could be scientifically modified for better pharmacological activity to establish safe and effective drugs (Rahman et al., 2009). Infectious diseases have been a life threatening problem for humans before antibiotics (Seyyednejad et al., 2010) and were important cause of morbidity and mortality among humans and account for about half of the death in tropical countries (Khosravi et al., 2006). With time infection rates have increased and antibiotic resistance has become an increasing therapeutic problem (Jarvis, 2008; Mitscher, 2008).

Chemotherapy has not achieved the much-required success in the eradication of microbial infection because of the antimicrobial resistance developed by most pathogenic microorganisms (Unlu et al., 2008). Bacterial infections are some of the most serious global health issues of present

century (Ananth et al., 2010), they are evolving numerous mechanisms to evade antimicrobial agents (Parekh and Chanda, 2007, Sharath K et al., 2016). There is a need to identify new and novel antimicrobial agents that would help in alleviating the problems of emerging resistant pathogens (Talib et al., 2010). Using the different antimicrobial and phytochemical constituents of medicinal plants for the treatment of microbial infections as possible alternative to chemically synthetic drugs to which many infectious microorganisms have become resistant (Akinpelu et al., 2006) is widely accepted. A better understanding of the ecological role for antibiotics and antibiotic resistance in non-clinical environments may eventually help to predict and counteract the emergence and future evolution of resistance (Martinez et al., 2008)

Fistula in ano is one of the commonest ailments pertaining to the ano-rectal area and is very difficult to cure by surgical intervention where the side effects like incontinence and high chance of recurrence are commonly experienced. Kshara sutra treatment is an effective way of curing fistula without side effect and least recurrences. Thus the kshara sutra may have antimicrobial activity and its chemical nature responsible for the pharmacology activities of the thread. Kshara sutra thread is prepared by

alkaline powder of the plant *Achyranthes aspera*, latex of *Euphorbia antiquorum* and *Curcuma longa*.

2. Methodology

2.1 Collection of latex

The plant specimen was identified and authenticated by department of Dravyaguna, Gampaha Wickramarachchi Ayurveda Institute. The latex was collected from plant by breaking up the stem of *Euphorbia antiquorum*. The latex was collected in the morning hours between 8 to 9 a.m. in to a glass container and maintained in an ice cold condition till the use of latex.

2.2 Sample preparation and test for antibacterial activity

Collected latex was extracted in different solvents; methanol, ethanol, and chloroform accordingly the extracts were prepared by dissolving 1.0 ml of latex in 9.0 ml of solvents respectively. The extracts were centrifuged at 3500 rpm for 5 minutes and the supernatant was collected. The collected crude extract was used for preparing discs for testing antibacterial sensitivity as per the method described by Benrit et al. (2012).

In the present study, the Gram positive bacteria: *Staphylococcus aureus* and *Streptococcus agalactiae*, and Gram negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa* were tested. The standard strains (American Type Culture Collection-ATCC), *Staphylococcus aureus*

(ATCC 25923), *Streptococcus agalactiae* (ATCC 12386), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) were obtained from stored samples at department of microbiology, faculty of medicine, University of Colombo.

The sterile filter paper discs were soaked with 100 µl of different latex extracts and were dried for 30 minutes. The prepared agar plates were seeded with each bacterial strain. Bacteria and the filter paper discs soaked with different extracts of the latex were arranged at regular intervals inside the petri dishes. The standard antibiotic disc was placed at the center of each plate. The plates were incubated overnight at 37°C in an incubator. Three replicates were maintained for each extract. The diameter of the zone of inhibition (ZOI) was measured in millimeters.

Amoxicillin 100µg/ml was used as positive control and Sterile distilled water was included as negative control

3. Results

E. antiquorum latex was used as a classical binding agent for the kshara sutra preparation. It was observed that latex has mean ZOI for the *S. aureus* 8.66 ± 0.21, and for *E. coli* 8.33 ± 0.21. There were no inhibition for the *S. agalactiae* and *P. aeruginosa*. In comparison to the standard antibiotic, relatively low ZOI was observed with latex. The significant difference was observed in ZOI for antibiotic and for latex that difference was statistically high significant (Table 1).

Table 1: ZOI for *Euphorbia antiquorum* latex and amoxicillin

Type of bacteria	Number of replicates	Mean ± SE for latex	Mean ± SE for Amoxicillin	t- value	Probability value
<i>S. aureus</i>	3	8.66 ± 0.21	30.23 ± 1.19	17.89	P<0.05 SIG
<i>E. coli</i>	3	8.33 ± 0.21	21.00 ± 0.45	23.24	P<0.05 SIG
<i>S. agalactiae</i>	3	6.00 ± 0.00	22.60 ± 0.92		
<i>P. aeruginosa</i>	3	6.00 ± 0.00	6.00 ± 0.00		

4. Discussion

Plant based products have been effectively proven for their utilization as source for antimicrobial compounds. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity (Mahesh and Satish, 2008). Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants (Behera and Misra, 2005; Govindarajan et al., 2006). In the present study, the methanol latex extracts of *E. antiquorum* showed the antibacterial activity against *S. aureus* and *E. coli* which supports the potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. The extract of latex of *E. antiquorum* was subjected to a preliminary screening for antimicrobial activity against pathogenic

bacteria. It was clear from the present results, latex extracts exhibited pronounced activity against the two tested bacteria namely *E. coli* and *S. aureus* rather than other tested bacteria (*S. agalactiae* and *P. aeruginosa*) in this study.

In kshara sutra preparation *E. antiquorum* latex is used as a binding agent, 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 ZOI exhibited by the extract against *S. aureus* justifies their use by traditional medicinal practitioners in the treatment of boils, sores and wounds (A.A.J.P. Kumara et al., 2016).

Kshara sutra therapy of fistula seems to make good use of chemical cauterization with a medicated thread. The binding agent of the thread *E. antiquorum* latex provides natural mild herbal base which performs the uniform and smooth cutting of normal tissue and the abnormal granulation as well and thereby reduces the depth of fistula with no or least recurrences (Kothia et al., 1986).

The diverse composition of the latex, which includes toxic compounds as well as other interesting and potentially bioactive molecules such as diterpenes and triterpenes

could be responsible for antibacterial and anti-fungal activity (Kupchan et al., 1976; Ferreira et al., 2002). In this study it was observed that *E. antiquorum* latex possess mild antibacterial property and our finding comply with previous study of (Sumathi et al., 2011).

5. Conclusion

Our study on methanolic extract of *E. antiquorum* latex revealed that the latex is potentially rich in antimicrobial compounds and suggests that they represent an economic and safe alternative for treatment of fistula in ano.

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Conflict of interest

None declared

6. References

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