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Full Length Research Article

In vitro Sensitivity Pattern of Selected Bacterial Pathogens on *Urginea indica* kunth. Rhizome Extract

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Abstract

The antibacterial activity of *Urgineaindica* ethanol, ethyl acetate, acetone, chloroform and petroleum ether solvent extracts were tested against ten pathogenic bacteria by disc diffusion method. Among the five solvents used ethanol extract exhibited greater inhibitory effect against *Klebsiellaoxytoca* followed by ethyl acetate, acetone and chloroform extracts against *Staphylocoocuslentus, Bacillus cereus* and *Staphylococcus lentus* respectively. Petroleum ether extract of the plant rhizome was not exhibited any inhibitory activity against all the tested organisms.

***Key words*:** Antibacterial activity, *Urgineaindica,* Disc diffusion assay, Infectious pathogens, Organic solvents

Infectious diseases seem to be the biggest cause of death worldwide, claiming the lives of about 60% of the population every day. Morbidity and mortality due to infections continues to be a major threat in many countries. Infections due to variety of bacterial pathogens such as *Escherichia coli* and *Staphylococcus aureus* are most common. In recent years, due to indiscriminate use of antibiotics, microorganism have become resistant especially drug resistance to human pathogenic bacteria have been commonly reported from all over the world. In addition, antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, immunosuppressant and allergic reaction, which has created immense clinical problems in the treatment of infectious [14]. Therefore, there is an urgent need to develop alternative antimicrobial drugs for the treatment of infectious diseases; one of the approaches is to screen local medicinal plants for possible antimicrobial properties. Medicinal plants continue to be a valuable resource in the fight against major diseases around the world. According to world health organization (WHO 1993), more than 80% of the world's population is dependent on the traditional medicine and a major part of the traditional therapies involves the use of plant extracts and their active constituents. However, scientific research into plant antibacterial active chemicals is a relatively young field. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries [16].

Urginea indica belonging to the family liliaceae, is a glabrous herb with polytypic genus consisting of about 99 species all over the world, 9 occurring in India. *U. indica* is reputed for a number of therapeutic benefits. The plant's bulb has traditionally been made as a supplier of therapeutic products, both pharmacological and biocidal. Squill bufadienolides being utilized as cardiotonic drugs. According to literature survey the extracts of *Urginea indica* bulbs have been reported for pharmacological and biological activities which are antifungal [12], [19] antioxidant [6], [13], gastrointestinal stimulant [17], anti-inflammatory, anti-carcinomic [1] cardio-tonic [5] antimicrobial, anti-insecticidal [11], anti-arthritic [9] and antidiabetic [7]. The plant extracts is also reported for dermatological, diuretic properties, abortifacient effects and effects on menstrual cycle, as cough expectorant, cure for renal failure, cronic rhinitis, cronic pulmonary disorder.

Hence, the present study was undertaken to evaluate the antibacterial activity of *U. indica* used in for the treatment of infectious diseases caused by microorganisms. Here, extracts of the *U. indica* was examined for its antibacterial potential against harmful bacterial pathogens.

MATERIALS AND METHODS

The plant material of *U. indica* was collected and the healthy plant parts were selected and it is allowed to shade dried

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for 5-8 days or until they broke by hand easily in the laboratory at room temperature. After drying the plant parts were ground to a fine powder by using an electronic blender and stored in a closed container at room temperature for further uses. 50 gms of the plant leaf and stem powdered material was separately impregnated with 300 ml of each of the solvents viz., ethanol, ethyl acetate, acetone, chloroform and petroleum ether and it was filtered through Whatman No.1 filter paper. The paste-like preparations are kept in pre-weighed screw - capped containers, and also the extract yield then estimated using the bottle's initial and final weights. The extracts were maintained in such glass vial vials in the fridge at 4 °C. Each of the extract was individually reconstituted by using minimal amount of the extracting solvent prior to use.

Test bacteria

The ten bacterial species were selected and the species were purchased from the Department of Microbiology, K. AP Viswanathan medical college, Tiruchirappalli, Tamil Nadu. The following gram-positive bacteria viz., *Staphylococcus lentus, Staphylococcus haemolyticus, Staphylococcus aureus* and *Bacillus cereus and* the gram-negative bacteria viz., *Escherichia coli, Serratia marcescens, Enterobacter amnigenus, Klebsiella pneumoniae, Klebsiella oxytoca* and *Brevibacterium paucivorans* were used for testing.

Disc preparation and sterilization

Whatman No.1 filter paper is the most convenient material for preparing the discs. The filter paper discs of uniform size are impregnated with the compound (plant extract) usually consisting of absorbent paper and sterilized in an autoclave.

Antibacterial activity test (Disc diffusion method)

The standard procedure was used for the antibacterial test. The sterile Nutrient Agar medium was poured (10-15 ml) into each sterile Petri plates. After solidification, 100 µl of suspension containing 10^8CFU/ml of each test bacteria were spread over Nutrient Agar plates. The sterile filter paper discs (6 mm in diameter) were impregnated with 10 µl of the 3 mg/ml extracts (30 µg/disc) placed on the inoculated agar. The similar solvents used to dissolve the plant extract have been used to make negative controls. Chloramphenicol (30 µg/disc) were used as positive reference control to determine the sensitivity of plant extract on each bacterial species. The inoculation plates were then incubated hours at 37 °C. The zones of inhibition against the tested microorganisms were measured to determine antibacterial efficiency.

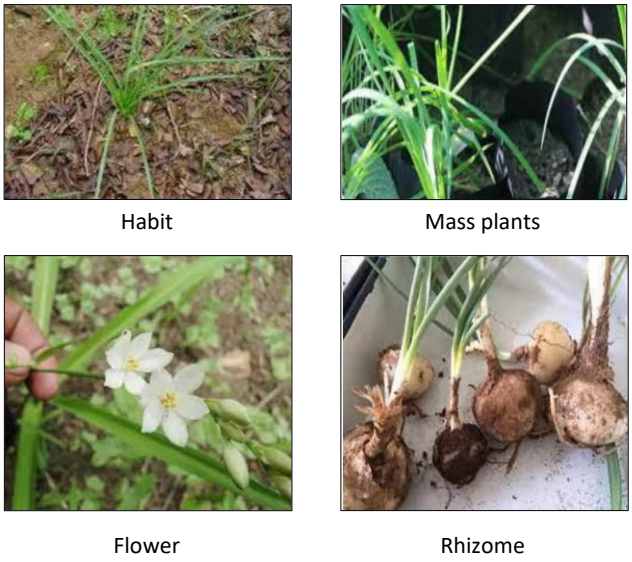

Fig 1 Habit of *Urginea indica*

RESULTS AND DISCUSSION

In vitro antibacterial activity of ethanol, ethyl acetate, acetone, chloroform and petroleum ether extracts of rhizome was evaluated by disc diffusion assay against clinical pathogenic bacteria. The bacteria include both gram–positive and gram–negative. Among the five extracts the ethanol, ethyl acetate and acetone, chloroform extracts have highest efficiency compared to petroleum ether extract. The ethyl acetate rhizome extracts showed greater inhibition than other solvent extracts.

Table 1 Antibacterial screening of rhizome extracts of *Urginea indica* (Kunth.) on pathogenic bacteria (Disc diffusion method)

Test bacteria	Ethyl acetate		Ethanol		Acetone		Chloroform		Petroleum ether		Positive control*
	E (30 µg/disc)	N	E (30 µg/disc)	N	E (30 µg/disc)	N	E (30 µg/disc)	N	E (30 µg/disc)	N	(30 mcg/disc)
Staphylococcus haemolyticus	9.33 ± 2.30	-	15.66 ± 1.15	-	15.66 ± 2.51	-	10.66 ± 2.51	-	-	-	15.3 ± 0.57
Staphylococcus lentus	8.66 ± 1.15	-	12 ± 1	-	15.5 ± 2.12	-	10 ± 1.73	-	-	-	20.6 ± 1.55
Staphylococcus aureus	9 ± 1.73	-	16 ± 3.60	-	15 ± 5	-	8.33 ± 0.57	-	-	-	10.6 ± 0.57
Bacillus cereus	12 ± 1	-	14 ± 1.73	-	11.33 ± 2.30	-	9 ± 1	-	-	-	9.0 ± 1.00
Salmonella typhi	8 ± 0	-	13.33 ± 2.51	-	7.66 ± 0.57	-	8 ± 0	-	-	-	13.3 ± 1.52
Eschericia coli	8.66 ± 1.15	-	16.33 ± 4.93	-	12 ± 2	-	-	-	-	-	19.0 ± 1.00
Serratiamarcescens	9.33 ± 2.3	-	9.33 ± 1.15	-	10.66 ± 1.15	-	9.33 ± 1.15	-	-	-	18.6 ± 1.51
Enterobacteramnigenous	9.66 ± 0.57	-	17.33 ± 0.57	-	12 ± 2.64	-	10 ± 1	-	-	-	19.6 ±0.57
Klebsiellapneumoniae	10.33 ± 3.21	-	9 ± 1	-	-	-	-	-	-	-	18.6 ± 1.51
Klebsiellaoxytoca	12.33 ± 5.13	-	11.66 ± 1.52	-	12.66 ± 3.78	-	8 ± 0	-	-	-	20.6 ± 1.15

'*' represents as Chloramphenicol,
'E' represents as 'Experimental',
'–' represents as 'no inhibition,
'N' represents as 'Negative control'

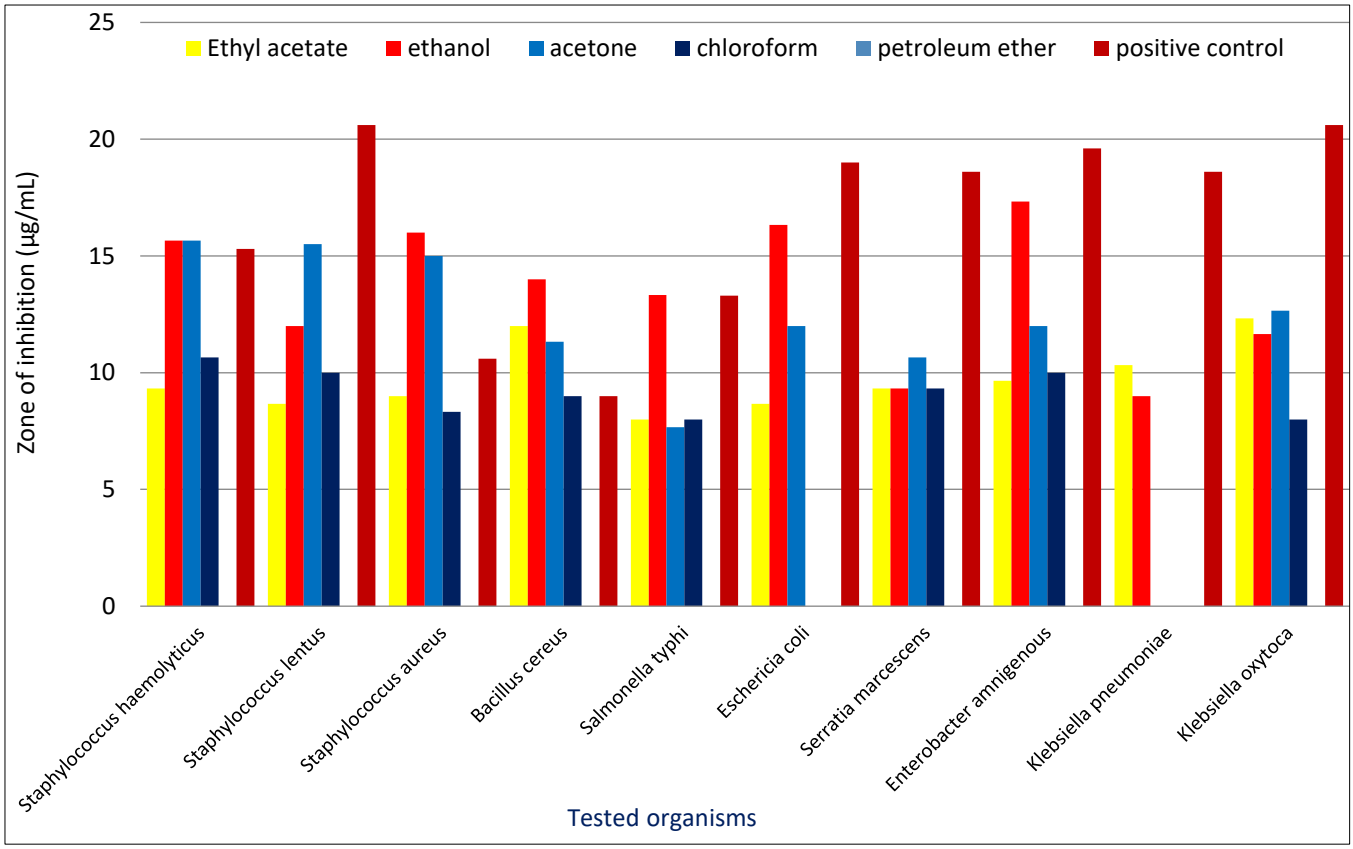

Fig 2 Antibacterial screening of rhizome extracts of *Urginea indica* (Kunth.) on pathogenic bacteria (Disc diffusion method)

The ethyl acetate, ethanol, acetone, chloroform and petroleum ether extracts of *Urginea indica* (rhizome) against both gram-positive and gram-negative bacteria by using the disc diffusion method is given in (Table 1). The in vitro findings were quantified in units of an inhibitory zone over each disc caused by antibacterial characteristics diffused into the surrounding media from the crude extracts-soaked disc. Among various solvent extracts tested ethanol rhizome extract exhibited high degree of inhibition followed by ethyl acetate, acetone and chloroform extracts demonstrated in (Table 1, Fig 2). The petroleum ether extracts did not show any antibacterial activity. In addition, the inhibition zones formed by standard antibiotic disc (chloramphenicol 30 mcg/disc) and those filter paper discs injected with ethanol, ethyl acetate, chloroform, petroleum ether, ethanol (1: 1) (negative controls) are also listed in (Table 1). The size of zones of inhibition in every sample was compared to the diameter of inhibition zones in common antibiotics. The rhizome extract exhibited highest zone on ethanol extract against *Enterobacteramnigenous* (17.33 ± 0.57 mm), *Eschericia coli* (16.33 ± 4.93 mm), *Staphylococcus haemolyticus* (15.66 ± 1.15) at same time the equal result was observed on acetone extract *Staphylococcus haemolyticus* (15.66 ± 2.51 mm), *Staphylococcus lentus* (15.5 ± 2.12 mm), *Staphylococcus aureus* (15 ± 5 mm). Moderate inhibition was noted on *Urginea indica* (rhizome) chloroform extract against *Bacillus cereus* (9 ± 1), *Serratia marcescens* (9.33 ± 1.15), *Klebsiella oxytoca* (9.33 ± 0.57). Whereas low degree of inhibition zones was associated with *Eschericia coli*, *Staphylococcus lentus* (8.66 ± 1.15 mm), *Staphylococcus aureus* (8.33 ± 0.57 mm) *Klebsiella oxytoca* (6.6 ± 5.7 mm) (Fig 2). It was surprising to note that *Staphylococcus aureus* and *Salmonella typhi* the multi-drug resistant bacteria even to synthetic antibacterial drugs were found to be sensitive to the ethanol and ethyl acetate extracts. Although other plant species viz. *Boswellia* [2], *Commiphora* [4], *Jatropa* [3], *Punica* [15], *Withania* [10], *Cissus* [8] and *Cleome* [18] have been subjected to several studies and antimicrobial activities have been found. The results obtained in the course of the present study are in agreement with the previous studies.

CONCLUSION

The antimicrobial screening of *Urginea indica* (rhizome) proved that the plant contains bioactive principles which exhibit significant antimicrobial activity against most of the pathogenic bacteria. The plant extract of *Urginea indica* (rhizome) had some measurable antibacterial sensitivity against the tested pathogenic bacteria. The ethyl acetate, ethanol and acetone extracts were the better extracting solvents than the chloroform and petroleum ether used for the extraction of antibacterial principles in this study which showed high degree of antibacterial response with regard to the inhibition of bacterial growth.

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