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Evaluation of antibacterial activity of leaf and stem extracts of *Oxystelma esculentum* R. Br., against selected pathogenic bacteria

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Abstract

Oxystelma esculentum R. Br., leaf and stem were extracted with five different organic solvents *viz*. ethanol, ethyl acetate, acetone, chloroform and petroleum ether and evaluating the antibacterial activity by disc diffusion assay against various human pathogenic bacteria. Chloramphenicol is used as the standard antibiotic. The result of the present study reveals, among the five solvents both leaf and stem extracts of ethyl acetate and ethanol shows significant results on more number of tested organisms. Ethanol leaf and stem extracts exhibits greater zone of inhibition on *Brevibacterium paucivorans*. The moderate level of inhibitions shown in maximum number of tested organisms.

Keywords: antibacterial activity, chloramphenicol, disc diffusion assay, human pathogens, *Oxystelma esculentum* R. Br

Introduction

Many of the developing countries were followed conventional clinical exercise as an essential part of their life style [12]. Several herbs were recognized to own medicinal property which includes anti-microbial effects [1]. Herbal vegetation of better flora may be a new source of antimicrobial retailers possibly with novel mechanisms of motion [6]. Plant extracts show off antimicrobial belongings because of presence of positive bioactive compounds, which if remotes can be utilized in pharmacological education without any facet effects. *Oxystelma esculentum* (L.F) R.Br. ex Schltes is an important medicinal plant which is used in the traditional systems of medicine for various ailments. It belongs to the family Asclepiadaceae, and which possess milky substance, so it was called as milky weed [13]. The plant has various pharmacological effects like diuretic, aphrodisiac, anthelmintic and bronchitis, useful in leucoderma and the fruit is expectorant, anthelmintic and the juice is used in gonorrhoea, muscle pain [10]. The milky sap of this plant used as a wash for ulcers. In combination with turpentine it is prescribed for itch. *Oxystelma esculentum* is reported to possess antiseptic, depurative and galactagogue properties. A decoction of the plant is useful as a gargle in infections of throat and mouth [13]. Due to the multi-drug resistant lines, bacterial infections are nonetheless a risk to public fitness [2, 11]. The capability of microorganism to adapt into new strains on non-stop publicity to synthetic tablets shifted the eye from synthetic control to herbal products [7]. The huge use of synthetic pills, immoderate unwanted remedy will purpose increasing aspect consequences in the frame, every so often, the toxic results produced via the administration of medicine is much extra a critical problem than that of the ailment it self [3]. Usually all medicinal preparations have been derived from flora, whether or not within the easy form of raw plant materials or in the delicate shape of crude extract mixtures and many others. Current estimates recommend that several hundreds of plant life had been known with medicinal applications in various systems [12].

About, 20 according to cent of the flowers located within the global have been submitted to pharmaceutical or biological checks and a sustainable variety of latest antibiotics added within the market are received from natural or semi synthetic assets [14]. Therefore, the present investigation is to evaluate the *in vitro* antimicrobial activity of the various solvent extracts of *Oxystelma esculentum* R.Br. against some gram positive and gram negative bacteria.

Materials and Methods

The plant material of *Oxystelma esculentum* was collected and the healthy plant parts were chosen carefully and removed an old insect damaged, fungus infected leaves, stems and roots.

The selected plant parts were allowed to shade dried in the laboratory at room temperature for 5-8 days or until they broke by hand easily.

After drying by using an electronic blender the plant parts were ground to a fine powder and the powders were stored in a closed container at room temperature for further uses. Fifty grams of the plant leaf powdered stem material was separately impregnated with 300 ml of each of the solvents *viz.*, ethanol, acetone, chloroform, ethyl acetate and petroleum ether at the end of 48hrs each extract was filtered through Whatman No.1 filter paper and filtrates were concentrated at room temperature.

The paste like extracts were stored in pre-weighed screw cap bottles and the yield of extracts was calculated based on initial and final weight of the container. These screw cap bottles with the extracts were kept in refrigerator at 4°C. Each of the extract was individually reconstituted by using minimal amount of the extracting solvent prior to use.

Tested bacteria

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

Disc preparation

The filter paper discs of uniform size are impregnated with the compound (plant extract) usually consisting of absorbent paper. It is most convenient to use Whatman No.1 filter paper for preparing the discs. Dried discs of 6 mm diameter were prepared from Whatman No.1 filter paper and sterilized in an autoclave. These dried discs were used for the test.

Antibacterial activity test (Disc diffusion method)

Sterile liquid Nutrient Agar medium was poured (10-15 ml) into each sterile petriplates. 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. Negative controls were prepared using the same solvents employed to dissolve the plant extract. Chloramphenicol (30 µg/disc) were used as positive reference control to determine the sensitivity of plant extract on each bacterial species. The inoculated plates were incubated at 37° C for 24 hrs. Antibacterial activity was evaluated by measuring the zones of inhibition against the test organisms. Each assay was conducted in triplicate.

Results and Discussion

In vitro antibacterial activities of ethyl acetate, ethanol, acetone, chloroform and Petroleum ether leaf and stem extracts of *Oxystelma esculentum* R. Br., was evaluated by disc diffusion assay against pathogenic bacteria (Table 1 & 2). Both leaf and stem extracts of ethyl acetate, ethanol and acetone reveals appreciable results in most of the bacteria. Ethyl acetate extracts exhibits better inhibitory effect when compare to other solvent extracts. Ethyl acetate leaf extract showed significant zone against *E.coli* (13±2) and *Bacillus cereus* (12.3±2.5), in all other organisms have possess same level of inhibition. Whereas ethyl acetate stem extract exhibited much greater inhibition on *Brivibacterium paucivorans* (14.3±3.5), *Bacillus cereus* (12±1) *Escherichia coli* (12.3±1.5), *Serratia marcescens* (11±1) and *Brivibacterium paucivorance* (11.3±2.0), *Staphylococcus aureus* (10.3±1.5).

The ethanol stem extract showed greater inhibition than leaf extract. Observation made from ethanol stem extract showed a highest activity against *Brivibacterium paucivorance* (16.3±3.5) *Enterobacter amnigeneus* (14±1) *Serratia marcescens* (14±3), *E.coli* (12.3±2.5) *Bacillus cereus* (13.6±4.1) *Staphylococcus aureus* (11±1) *and Staphylococcus lentus* (10.3±1.5). In contrast with our results ethanol leaf extracts exhibits highest concentrations tried 75 and 100 µl/l exhibited the maximum antimicrobial activity against Escherichia coli (21.44 mm) [4]. Whereas, ethanol leaf extract showed a highest activity against *Enterobacter amnigeneus* (20±2.6), *Brivibacterium paucivorance* (17±3.6), *Serratia marcescens* (14±4.5), *E.coli* (12.3±4.5) *Klebsiella oxytoca* (11±4). The moderate inhibition zones were observed on stem extract against *Staphylococcus aureus* (8±1) and *Bacillus cereus* (7±1). *Klebsiella pneumonia.*is more resistant on both leaf and stem ethanol extracts. Likewise, ethanol extract of *Calotropis procera* leaves showed greater zone of inhibition compared with chloroform and aqueous extracts against most of the tested organisms [9]. The Acetone stem extract results were showed greater than leaf extract. The inhibition zones observed higher to Moderate in both acetone leaf and stem extract. The significant zones were noted against *E.coli* (14.6±1.5) *Enterobacter amnigenus* (13±2.6) *Bacillus cereus* (13±3) *Bivibacterium paucivorance* and *Serratia marcescens* (12±2) on stem extract. At same time in leaf extract showed moderate inhibition against most organisms (Table-1). The chloroform leaf extracts showed greater inhibition activity compare to stem extract. Likely, *Staphylococcus aureus* and *E.coli* was more sensitive to the chloroform extract of *Leptadenia reticulate* [8]. In petroleum ether leaf extract were showed optimum results only on three tested bacteria *viz*, *Staphylococcus heamolyticus* (7.6±1.1), *Staphylococcus aureus* (7.3±0.5) and *klebsiella pneumoniae* (7±1). No results were found in Petroleum ether stem extract against all tested bacteria (Table-1 and 2). (Figure - 1 and 2). According to Savitha and Balamurugan, 2016, chloroform, acetone, petroleum ether and ethyl acetate leaf extracts of *Oxystelma esculentum* exhibits highest zone of inhibition against *Staphylococcus aureus* [5].

Conclusion

This study was concluded that the ethanol and ethyl acetate leaf and stem extracts of *Oxystelma esculentum* R. Br., had high potential antibacterial activity against most of the organisms and also exhibits greater inhibition on many of the tested organisms. Acetone and Chloroform leaf and stem extracts showed moderate results in most of the tested bacteria. Whereas in petroleum ether leaf extract shown optimum results on only three organisms, there is no result in stem extract. Based on these results, *Oxystelma esculentum* had an efficiency to cure most of the bacterial diseases.

Table 1: Antibacterial screening of leaf extracts of *Oxystelma esculentum* Cav. on pathogenic bacteria (Disc diffusion method)

Test bacteria	Inhibition zone diameter in mm (mean ± SD)										
	Ethyl acetate		Ethanol		Acetone		Chloroform		Petroleum ether		Positive control
	E	N	E	N	E	N	E	N	E	N	
Gram-positive											
Staphylococcus heamolyticus	-	-	-	-	-	-	-	-	7.6±1.1	-	28 ± 0
Staphylococcus lentus	11.6±1.5	-	7.6±2	-	9.6±2.5	-	-	-	-	-	23 ± 0
Staphylococcus aureus	11±2.6	-	8±1	-	-	-	8±1	-	7.3±0.5	-	26± 0.5

Bacillus cereus	12.3±2.5	-	7±1	-	-	-	10.3±1.5	-	-	-	21 ± 0
Gram-negative											
Escherichia coli	13±3	-	12.3±4.7	-	12±6.8	-	11±2	-	-	-	25 ± 0
Serratia marcescens	11±1	-	14±4.5	-	6±5.2	-	10.3±1.5	-	-	-	28 ± 0
Enterobacteraerogens	13±2	-	20±2.6	-	8±1.5	-	8.6±2	-	-	-	24±1
Klebsiella pneumonia	-	-	-	-	-	-	-	-	7±1	-	23 ± 0
Klebsiella oxytoca	11.3±1.5	-	11±4	-	-	-	-	-	-	-	23 ± 0.5
Brevebacteriumpaucivorans	11.6±1.5	-	17±3.6	-	10.6±1.1	-	13.6±1.5	-	-	-	30 ± 0

'-' No inhibition; 'E'-Experiment; 'N'-Negative control; Positive control-Chloramphenicol (30mg/disc)

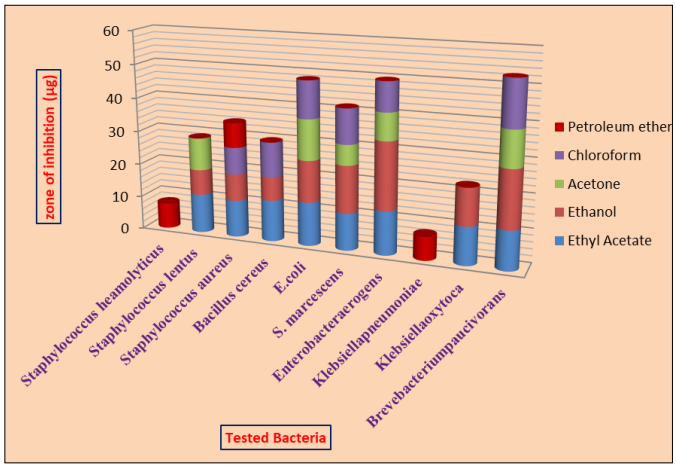

Fig 1: Antibacterial screening of leaf extracts of *Oxystelma esculentum* Cav. on pathogenic bacteria (Disc diffusion method)

Table 2: Antibacterial screening of stem extracts of *Oxystelma esculentum* Cav. on pathogenic bacteria (Disc diffusion method)

Inhibition zone diameter in mm (mean ± SD)											
Test bacteria	**Ethyl acetate**		**Ethanol**		**Acetone**		**Chloroform**		**Petroleum ether**		**Positive Control**
	E	**N**	**E**	**N**	**E**	**N**	**E**	**N**	**E**	**N**	
Gram-positive											
Staphylococcus heamolyticus	-	-	-	-	-	-	-	-	-	-	28 ± 0
Staphylococcus lentus	10.3±1.5	-	10.3±1.5	-	11±1	-	-	-	-	-	23 ± 0
Staphylococcus aureus	4.6±4	-	11±1	-	7±1	-	-	-	-	-	26± 0.5
Bacillus cereus	12±1	-	13.6±4.1	-	13±3	-	11.6±1.5	-	-	-	21 ± 0
Gram-negative											
Eschechia coli	12.3±1.5	-	12.3±2.5	-	14.6±1.5	-	10±1	-	-	-	25 ± 0
Serratia marcescens	11±1	-	14±3	-	12±2	-	11.3±1.5	-	-	-	28 ± 0
Enterobacter amnigenus	11.3±1.5	-	14±1	-	13±2.6	-	-	-	-	-	24±1
Klebsiella pneumonia	-	-	-	-	-	-	-	-	-	-	23 ± 0
Klebsiella oxytoca	-	-	-	-	-	-	-	-	-	-	23 ± 0.5
Brivibacterium aucivorans	14.3±3.5	-	16.3±3.5	-	12±2	-	-	-	-	-	30 ± 0

'-' No inhibition; 'E'-Experiment; 'N'-Negative control; Positive control-Chloramphenicol (30mg/disc)

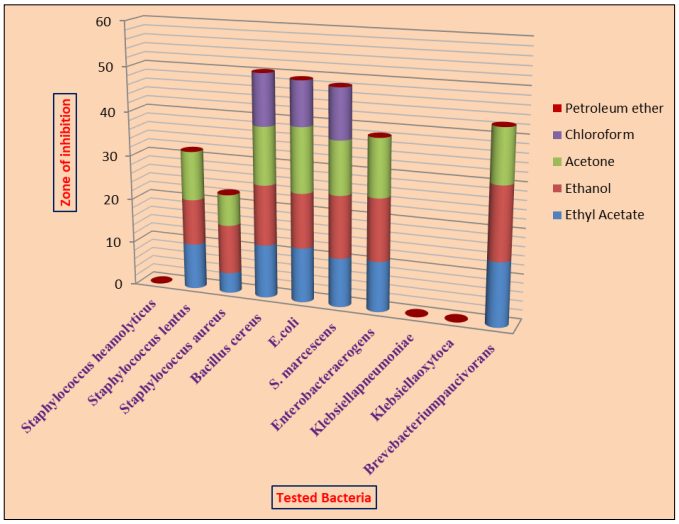

Fig 2: Antibacterial screening of stem extracts of *Oxystelma esculentum* Cav. on pathogenic bacteria (Disc diffusion method)

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