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In vitro antimicrobial activity of crude and chromatographic fractions of *Oxystelma esculentum* (L.F) R.Br. ex Schltes

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Abstract

The crude and chromatographic fractioned ethanolic extracts of *in vivo* plant and *invitro* callus of *Oxystelma esculentum*. were tested against *Escherchia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae* and fungi like *Candida albicans, Aspergillus niger, Cryptococcus neoformans, Mucor sp* using the agar well diffusion method. Both the *invivo* plant and *invitro* callus extracts inhibited the growth of RTI pathogens of both bacteria and fungi with zone of inhibition between 1.2 – 26.4 mm. The ethanol extract of stem callus of *Oxystelma esculentum* showed high activity (26.4mm and 16.9mm) against *E.coli and C. neoformans* respectively. The study showed that the plant extracts could be used as first line antimicrobials for the effective management of RTI.

Keywords: *Oxystelma esculentum*, antimicrobial activity, *in vitro* callus, Column chromatography fractions

Introduction

Traditional medicines based mostly on medicinal plants have been used for the treatment of various diseases by mankind for centuries. Herbs are also well-known to be the rich sources of biologically active compounds. Hence, one approach that has been used for the invention of antimicrobial agents from natural sources is based on the evaluation of traditional plant extracts (Priyanka *et al*., 2014). Currently, drug resistance to human pathogenic bacteria has been commonly and widely reported in literature (Mulligen *et al*., 1993; Davis 1994; Robin *et al*., 1998). Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines (Essawi and Srour 2000). Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Saxena, 1997; Nimri *et al*., 1999; Saxena and Sharma 1999).

Oxystelma esculentum (L.F) R.Br. ex Schltes is an important medicinal plant belonging to the family Asclepiadaceae used in the traditional systems of medicine for various ailments. The plant is hot, bitter, tonic, expectorant, pungent, dry and indigestible; causes flatulence, diuretic, laxative, aphrodisiac, anthelmintic, useful in leucoderma and bronchitis. The juice is used in gleet, gonorrhea, pain in the muscles, cough and given to children as an astringent. The milky sap forms 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 (Poornima *et al*., 2009). The aim of the present work was to evaluate the antimicrobial potentiality of the crude and CC fractioned *in vivo* plant and *in vitro* callus extracts of *O. esculentum* against the growth of RTI pathogens.

Materials and Methods

Collection of plant materials

The plant parts of stem and leaf were collected from mature plants and washed with water and then chopped into small fragments. The stem callus and leaf callus were collected from culture tubes. The materials were then shade dried at ambient temperature (32°C) for 10 to 15 days and the drying operation was carried out under controlled conditions to avoid chemical changes. The dried samples were crushed into fine powder using an electronic blender. The powdered samples were stored in polythene containers at room temperature.

Preparation of plant extracts using Soxhlet apparatus

The powdered samples were extracted by using soxhlet apparatus at 47^0C ethanol, chloroform and acetone were used as a solvent. After extraction, the extract was dried at 50^0C in hot air oven. Extracted samples were stored at properly and can be used for antimicrobial activity against RTI

Chromatographic fractions

The crude ethanolic extract of the dried plant tissue and callus material were reconstituted in absolute ethanol and spotted on analytical TLC and the following solvent system i.e., ethanol/ ethylacetate, 6:4. After separation, the TLC plate was exposed to iodine fumes in a chamber.

Column separation of the extracts was carried out with a glass column of internal diameter 80 mm and length 100 cm. Sufficient quantity of a column grade silica gel (120 - 200 mesh size) was wet-packed using benzene/ ethylacetate (6:4) solvent system. A 10 g amount of the crude extract was first dissolved in 20 ml of ethanol, and then mixed with about 20 g of the silica gel to become slurry. The slurry was loaded onto the wet packed column and continuously eluted with the mobile phase (ethanol/ ethylacetate, 6:4). Approximately 20 ml aliquots of eluent were collected while observing the distance traveled by the sample down the column; in addition, bands of the same sample formed on TLC were also monitored.

Antimicrobial activity

The bacteria, *Escherchia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and fungi, *Candida albicans*, *Aspergillus niger*, *Cryptococcus neoformans*, *Mucor sp* were used for the experiment.

Antibacterial activity was carried out using well-diffusion method (Perez *et al*., 1990). Petri plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA) (Hi-media, Mumbai) for bacteria. The test cultures (10^5 dilution) were swabbed on the top of the solidified media and allowed to dry for 10 min. After solidification of media, wells were made in the seeded plates with the help of a sterile well borer (6 mm dia.). Well are filled with 25, 50, 75 and 100µl of the ethanolic and CC fractions of stem, leaves, stem callus and leaf callus extract and growth inhibition zones were measured after 24 h of incubation at 37^0C. Zone of inhibition was recorded in millimeters and the experiment was repeated twice.

The same method as for bacteria was adopted. Instead of nutrient agar, potato dextrose agar was used. The inoculated medium was incubated at 25ºC for two days for the *Candida albicans* and three days for other fungi tested. Zone of inhibition was recorded in millimeters and the experiment was repeated thrice.

Results and Discussion

Antibacterial activity of ethanol extracts of leaf stem, callus and chromatographic fractioned extracts were tested against RTI bacteria like *Escherchia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and fungi like *Candida albicans*, *Aspergillus niger*, *Cryptococcus neoformans*, *Mucor sp*. Four different concentrations (25, 50, 75 and 100 µl/l) of ethanol and CC fractions showed varying degree of inhibitory effect.

The results showed that the ethanol extract of *Oxystelma esculentum* by silica gel column chromatography was active against all RTI pathogens investigated, as supported by other researchers who reported that *Prinsepia utilis* was found to be active against Gram-positive bacteria and Gram-negative bacteria (Zhang *et al*., 2007)

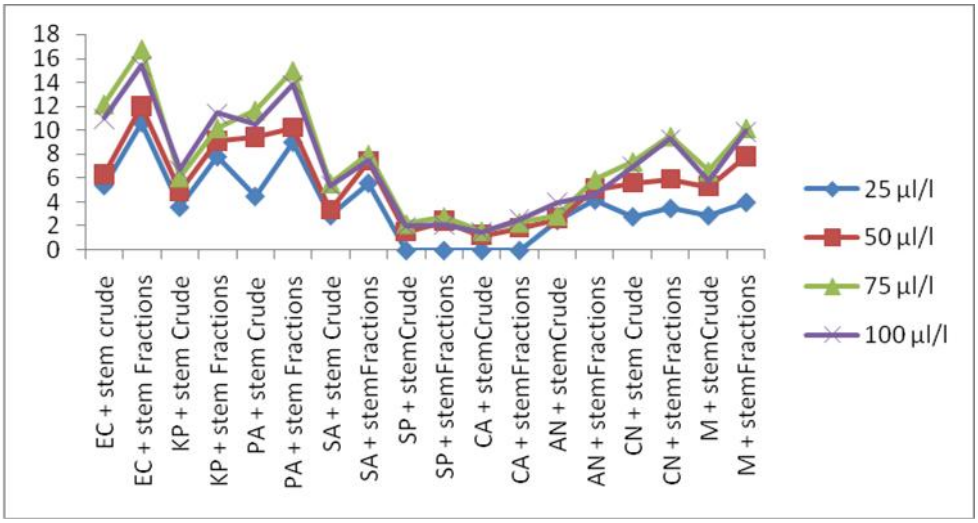
In vivo plant parts

The ethanol extract of the leaves was active against both RTI bacteria and fungi with inhibition (Table 1). Out four concentrations, 25 µl/l of both extracts did not show any antibacterial activity against *Pseudomonas aeruginosa* (40 µl/l and 60 µl/l in crude extract also) and *A niger* (40 µl/l in crude extract also). The next higher concentration 50 µl/l displayed the desired antibacterial activity against both bacteria and fungi. The highest concentrations tried 75 and 100 µl/l exhibited the maximum antimicrobial activity against *Escherichia coli* (21.44mm and 20.0 mm respectively) and *C. neoformans* (17.8mm and18.3 mm). Besides, *Escherchia coli* (16.8mm) is very sensitive at 75µl/l dose and 20 µl/l concentration of stem extract displayed inactivity against *Proteus mirabilis*, *Pseudomonas aeruginosa* (60 µl/l also) and *S. pneumoniae* and *C. albicans*. In the present study indicated that the antibacterial activity of active fractions separated from the ethanol extract of *Oxystelma esculentum* against bacteria and fungi were better compared with crude extracts tested.

Table1: Antimicrobial activity of ethanolic extracts of leaf and its CC fractions against RTI pathogens

S.No	Name of bacteria	Sample	Zone of inhibition in mm			
			25 µl/l	50	75	100
Bacteria						
1.	*E.coli*	Crude	6.2	20.4	19.2	15.6
		Fractions	10.0	25.9	21.4	20.0
2.	*K. pneumoniae*	Crude	2.8	12.1	14.3	13.7
		Fractions	5.1	16.6	14.8	14.0
3.	*P. aeruginosa*	Crude	0.0	0.0	0.0	1.5
		Fractions	0.0	1.2	1.8	3.8
4.	*S. aereus*	Crude	5.7	10.3	10.8	15.4
		Fractions	5.6	9.8	14.6	17.2
5.	*S. pneumoniae*	Crude	2.0	6.4	10.3	13.5
		Fractions	4.1	8.5	13.9	16.8
Fungi						
6	*C. albicans*	Crude	1.5	3.3	8.3	7.1
		Fractions	2.0	4.8	11.0	12.5
7	*A niger*	Crude	0.0	0.0	1.6	2.0
		Fractions	0.0	1.1	2.8	2.5
8	*C.neoformans*	Crude	2.3	4.7	12.0	11.2
		Fractions	2.9	5.9	17.8	18.3
9	*Mucor sp*	Crude	1.8	2.3	3.4	5.7
		Fractions	3.1	3.5	4.8	5.1

Fig1: Antimicrobial activity of ethanolic extracts of stem and its CC fractions against RTI pathogens



In vitro callus

The ethanol extract of leaf callus showed different antimicrobial activity towards the test organisms of RTI pathogens. The ethanol extract of leaf callus of *Oxystelma esculentum* showed low activity against *S. pneumoniae* and *C. albicans* at 25 µl/l, high activity (22.0mm and 17.0mm) against *E.coli* and *C. neoformans* respectively (Fig 2). On the other hand, the ethanol extract of stem callus of *Oxystelma esculentum* showed low activity against *P. aeruginosa* and *Mucor sp* at 25 µl/l, high activity (26.4mm and 16.9mm) against *E.coli* and *C. neoformans* respectively .

Fig 2: Antimicrobial activity of ethanolic extracts of leaf callus and its CC fractions against RTI pathogens

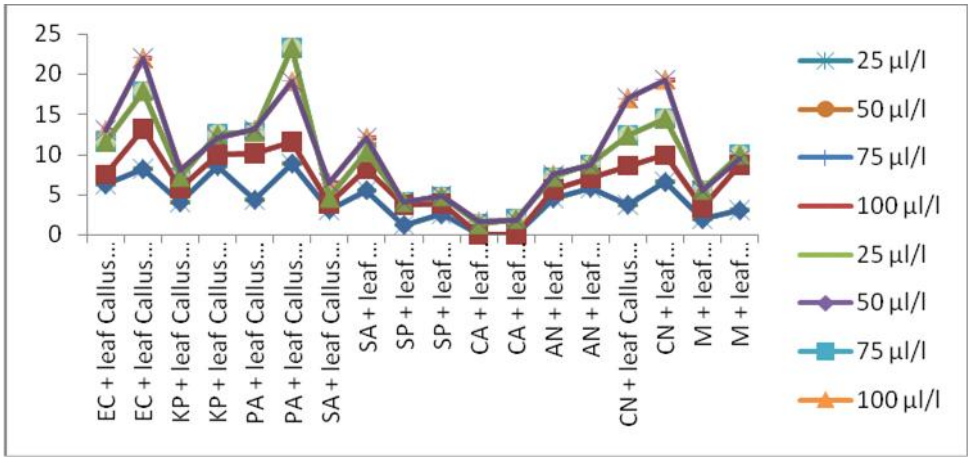


Table 2: Antimicrobial activity of ethanolic extracts of stem callus and its CC fractions against RTI pathogens

S.No	Name of bacteria	Sample	Zone of inhibition in mm			
			25 µl/l	50 µl/l	75 µl/l	100 µl/l
Bacteria						
1.	*E.coli*	Crude	2.8	2.9	6.4	6.0
		Fractions	6.6	14.9	26.4	20.8
2.	*K. pneumoniae*	Crude	3.3	6.0	5.4	7.5
		Fractions	8.3	14.2	17.9	12.2
3.	*P. aeruginosa*	Crude	0.0	1.8	2.9	2.2
		Fractions	0.0	2.6	6.0	5.0
4.	*S. aereus*	Crude	4.7	3.7	5.8	6.0
		Fractions	7.7	12.0	22.4	20.8
5.	*S. pneumoniae*	Crude	5.3	8.9	9.9	5.5
		Fractions	8.4	10.6	17.0	10.3
Fungi						
6	*C. albicans*	Crude	1.5	4.3	5.9	6.3
		Fractions	1.8	6.6	14.3	10.6
7	*A niger*	Crude	2.5	1.7	3.4	4.5
		Fractions	3.0	7.4	12.7	12.2
8	*C.neoformans*	Crude	2.6	6.3	8.6	7.9
		Fractions	2.8	12.3	16.9	15.8
9	*Mucor sp*	Crude	0.0	1.3	1.6	2.0
		Fractions	1.8	2.9	2.4	2.9

Compared to *invivo* plant parts, the callus had better activity against all bacteria and fungi. Similar reports of higher antibacterial activity of callus extracts of *Solidago virgaurea* against *P. mirabilis* (Thiem and Goslinska, 2002) support the present study. Meanwhile, based on the results obtained from the antimicrobial activity, these results further indicated *Oxystelma esculentum* had broad application prospects in antimicrobial plant agents against RTI. However, which particular active ingredient was poorly understood, there is a need for further to study and research.

References

Davis, J. 1994. Inactivation of antibiotic and the dissemination of resistance genes. Science, 264: 375- 382.

Mulligen, M.E., Murry-Leisure, K.A., Ribner, B.S., Standiford, H.C., John, J.F., Karvick, J.A., Kauffman, C.A. and Yu, V.L. 1993. Methicillin resistant *Staphylococcus aureus*. *The American Journal of Medicine*. 94: 313-328.

Nimri, L.F., Meqdam, M.M. and Alkofahi, A. 1999. Antibacterial activity of Jordanian medicinal plants. *Pharmacologycal Biology*. 37(3): 196-201.

Perez, G.R.M., Avila, J.G., Zavala, M.A., Perez, G.S. and Perez, G.C. 1990. *In vitro* antibacterial activity of *Loeselia mexicana* and *Croton ehrenbergii*. *Phytomed*., 3: 186.

Poornima, N., Umarajan, K.M. and Babu, K. 2009. Studies on Anatomical and Phytochemical Analysis of *Oxystelma esculentum* (L.f.) R.br. Ex Schltes. *Botany Research International*, 2 (4): 239-243.

Priyanka, A., N. Yogananth, M. Syed Ali and V. Anuradha, 2014. Antibacterial and antifungal activity of *Solanum torvum* Sw: An Ethnomedicinal Plant, *International journal of comprehensive research in biological sciences*, 1(3):6-12.

Robin, E.H., Anril, W., Alexander, M., Loeto, M. and Keith, K. 1998. Nasopharyngeal carriage and antimicrobial resistance in isolates of *Streptococcus pneumoniae* and *Haemophilus influenzae* Type b in children under 5 years of age in Botswana. *International Journal of Infectious Diseases*. 3(1): 18-25.

Saxena, K. 1997. Antimicrobial Screening of Selected Medicinal Plants from India. *Journal of Ethnopharmacology*. 58(2): 75-83.

Saxena, V.K. and Sharma, R.N. 1999. Antimicrobial activity of essential oil of *Lankana aculeata*. *Fitoterapia*. 70(1): 59-60.

Thiem, B. and Goslinska, O. 2002. Antimicrobial activity of *Solidago virgaurea* L. from *in vitro* cultures. *Fitoterapia*. 73: 514-516.

Zhang, R.J., Chui, B.Y., Zhao, J., *et al*. 2007. Effects of Extracts from *Prinsepia utilis* Royle on Antimicrobial Activity. *Journal of Anhui Agricultural Sciences*. 2: 408-409, 411.

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