Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(1): 447-451

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Preliminary phytochemical and antimicrobial activities of different solvent leaf extracts of *Oxystelma esculentum* R.Br.

G. Savitha and S. Balamurugan

Department of Botany, Annamalai University, Annamalainagar, Tamilnadu, India

---

ABSTRACTS

Oxystelma esculentum distributed in the southern part of India particularly in Tamilnadu has potential medicinal properties and it is used as diuretic, anthelmintic, oxytocic and laxative. Phytochemical screening of leaves extract revealed the presence of alkaloids, tannins, steroids, saponins, terpenoids, flavanoids, glycosides and phenolic compounds. The aim of this study was to screen the phytochemicals present in the leaf of Oxystelma esculentum and further analysis of the components present in antimicrobial activities. The microorganisms gram positive and gram negative bacterial viz. Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pesudomonas aeruginosa and Proteus vulgaris and fungal viz. Aspergillus niger, Aspergillus flavus and Candida albicans. The extracts of height activities of Staphylococcus aureus.

Key words: *Oxystelma esculentum* antimicrobial activities MIC, MBC, MFC.

INTRODUCTION

Natural products are defined as natural sources-derived sub-stances having biological activities. Natural products have long been implemented as alternative health care treatment and in discovery of modern drugs [1].

India has a rich flora that is widely distributed throughout the country. Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. Several plant species are used by many ethnic groups for the treatment of various ailments ranging from minor infections to skin diseases, diuretic, laxative, spermatogenetic, antitussive, anthelmintic and antileprotic, antiseptic, antiulcer useful in leucoderma and bronchitis of other indications [2]. In this screening we have studied the antimicrobial activity of dichloromethane: methanol (1:1, v/v) extract of 61 plant species against a battery of microorganisms including Gram positive and Gram- negative bacteria and fungi.

EXPERIMENTAL SECTION

Oxystelma esculentum (leaves, stem and roots) were collected from Annamalai University campus, Annamalainagar, chidambaram (latitude 11°23′17 n; longitude 79°42′57 E) Cuddalore district, Tamilnadu, India during month of June, 2013. Herbarium was deposited in department of botany, Annamalai University, (voucher specimen No. AUBOT#256). The collected specimens were washed with tap water, then surface sterilized with 10 per cent sodium hypochlorite solution, rinsed with sterile distilled water and allowed to shade dried under room temperature. The samples were ground into fine powder using an electric blender.

PHYTOCHEMICAL ANALYSIS

The different extracts of *Oxystelma esculentum* were used for qualitative phytochemical studies like alkaloids, cardiac glycosides, terpenoids, steroids, flavonoids, phenolic compounds, tannins and saponins [3]. In the present study, the phytochemical screening was studied with petroleum ether, chloroform, ethyl acetate, acetone and methanol extract of the leaves of *Oxystelma esculentum*. The results revealed, methanol leaf extracts of *Oxystelma esculentum* recorded the presence of alkaloids, terpenoids, steroids, flavonoids, phenolic compounds, tannins and saponins, followed by other extracts (**Table.1**) Phytochemical constituents such as tannins, flavonoids and several other aromatic compounds or secondary metabolites of plants serve as defense mechanism against predation by many microorganisms. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenolic compounds, saponins and steroids [4]. The presence of alkaloids, saponins, flavonoids, phenolic compounds, tannins, steroids and terpenoids in the leaf extract are very important and are used in analgesic, anti plasmodic and bactericidal activities [5]. Thus the preliminary screening test may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.

Table.1 Preliminary Phytochemical analysis of leaves extracts of *Oxystelma esculentum* R.Br.

S .No	Phytoconstituents	Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol
1.	Alkaloids	++	+	-	+	++
2.	Flavonoids	-	-	++	-	++
3.	Glycosides	-	-	-	+	-
4.	Phenolics	++	++	-	+	++
5.	Saponins	-	-	-	+	++
6.	Steroids	-	_	+	+	+
7.	Tannins	-	-	++	-	+
8.	Tepenoids	-	++	++	+	++

(++) = strong; (+) = positive (Present); (-) = Negative (absent)

PREPARATION OF THE EXTRACT

To prepare the methanol extracts, 100gm of each of the plant material was collected dried in the oven at 700C for 4 hr and reduced to powder. It was separately macerated with methanol, allowed to stand for 72hr and then filtered, dried extracts were stored in labeled sterile screw capped at 50C in the refrigerator, until when required for use.

MICROORGANISMS USED

The microorganisms used in the studies of human pathogenic bacteria viz. *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pesudomonas aeruginosa* and *Proteus vulgaris* typhi and fungal viz. *Aspergillus niger, Aspergillus flavus* and *Candida albicans*. Were obtained from stock culture in the Department of microbiology, Raja Muthiah Medical College & Hospital, and Annamalai University.

ANTIMICROBIAL ACTIVITY

The development of microbial resistance to presently avail-able antibiotics led the search for new antimicrobial agents [6]. Due to the problem of microbial resistance to antibiotics,attention is given toward biologically active components iso-lated from plant species commonly used as herbal medicine,as they may offer a new source of antimicrobial activities [7].

DISC DIFFUSION ASSAY

The antimicrobial activities of crude extracts of *Oystelma esculentum* was determined by disc diffusion method according to [8]. 20 mL of MHA/SDA was poured into petridishes and allowed to solidify. Plates were dried and 0.1 mL of standardized inoculum suspension (bacteria/fungi) was inoculated on the entire agar surface. The disc with different concentrations of crude extracts were prepared and aseptically applied on the surface of the petriplates with sterile forceps and gently pressed to ensure contact with the inoculated agar surface. Ciprofloxacin (5 µg/disc) for bacteria and Amphotericin-B (100 units/disc) for *Candida,* Ketoconazole (10µg/dics) for *Aspergillus* were used as positive controls. 10 per cent DMSO was used as blind control in these assays. Finally, the inoculated plates were incubated at 37°C for 24 h for bacteria, at 28°C for 24-48 h for *Candida* sps. And at 30°C for 72-96 h for *Aspergillus* sp. The zones of inhibition was observed and measured in millimeters. Each assay in these experiments was repeated three times.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

The minimum inhibitory concentration was determined in MHB for bacteria and SDA for fungi described by broth macro dilution method [9]. The plant extracts were dissolved in 10 per cent DMSO to obtained 2 mg/mL. 0.5 mL of stock solution was incorporated into 0.5 ml of MHB to get a concentration of 1000 to 15.62 µg/mL. 50 µL of standardized suspension of the test organism was transferred into each tube. The control tube contained only organism and devoid of plant extracts. The culture tubes were incubated at 37°C for 24 h for bacteria, 28ºC for 24-48 for *Candida* sps. And 30°C for 72-96 h for *Aspergillus* sp. The lowest concentrations, which did not show any growth of tested organism after macroscopic evaluation were determined as MIC.

MINIMUM BACTERIAL CONCENTRATION (MBC) AND MINIMUM FUNGICIDAL CONCENTRATION (MFC)

The MBC and MFC of the extracts were determined [10], by plating 100 µL of sample from each MIC assay tube with growth inhibition into freshly prepared MHB and SDA and the plates were incubated at 37°C for 24 h (Bacteria) and 28°C for 48-72 h (Fungi). The MBC and MFC values were recorded at the lowest concentration of the extracts that did not permit any visible bacterial/fungal colony growth on the agar plate during the period of incubation.

RESULTS AND DISCUSSION

In the present investigation, different solvent extracts of *Oxystelma esculentum* showed varied levels of antimicrobial activity **(Tables 2)** against the studied bacterial and fungal pathogens. The mean zone of inhibition produced by all the extracts ranged from 7.1±0.57 to 23.5±0.25 mm. The MIC, MBC and MFC values were between 15.62, and 1000 µg/ml. The methanol extract of *Oxystelma esculentum* showed good antibacterial activity with the highest mean zone of inhibition (23.5±0.25 mm) against *Staphylococcus aureus*, lowest MIC (15.62 µg/ml) and MBC (31.25 µg/ml) against *Staphylococcus aureus, Bacillus cereus* and *Bacillus subtilis* and methanol extract of *Oxystelma esculentum* showed antifungal activity with the highest mean zone of inhibition ranged from (12.8±0.28 mm) against *A.flavus*, lowest MIC (250 µg/ml) and MFC (500 µg/ml) against *A.nigar* and *A. flavus.* The ethyl acetate extract of *Oxystelma esculentum* showed good antibacterial activity with the highest mean zone of inhibition (19.7±0.35 mm) against *Staphylococcus aureus,* lowest MIC (31.2 µg/ml) and MBC (62.5 µg/ml) against *Staphylococcus aureius*, *Bacillus cereus* and *Bacillus subtilis* and ethyl acetate extract of *Oxystelma esculentum* showed antifungal activity with the highest mean zone of inhibition ranged from (12.0±0.03 mm) against *A.niger*, lowest MIC (250 µg/ml), and MFC (500 µg/ml) against *A. niger* and *A. flavus.* The acetone of *Oxystelma esculentum* showed good antibacterial activity with the highest mean zone of inhibition (17.8±0.25 mm) against Staphylococcus aureus, lowest MIC (62.5 µg/ml) and MBC (125 µg/ml) against *Staphylococcus aureus* and *Bacillus cereus* and acetone extract of *Oxystelma esculentum* showed antifungal activity with the highest mean zone of inhibition ranged from (12.0±0.30 mm) against *A.niger*, lowest MIC (250 µg/ml) and MFC (500 µg/ml) against *Candida albicans, A. niger* and *A. flavus*. The chloroform extract of *Oxystelma esculentum* showed good antibacterial activity with the highest mean zone of inhibition (15.8±0.30 mm) against *Staphylococcus aureus*, lowest MIC (62.5 µg/ml) and MBC (125 µg/ml) against Staphylococcus aureus and Bacillus cereus and chlorofrom extract of Oxystelma esculentum showed antifungal activity with the highest mean zone of inhibition ranged from (11.8±0.26 mm), lowest MIC (500 µg/ml) and MFC (1000µg/ml) against *Candida albicans A. niger*, and *A. flavus*. The petroleum ether extract of *Oxystelma esculentum* showed good antibacterial activity with the highest mean zone of inhibition (15.3±0.35 mm) against *Staphylococcus aureus*, lowest MIC (125µg/ml) and MBC (250 µg/ml) against *Staphylococcus aureus* and *Bacillus cereus* and petroleum ether extract of *Oxystelma esculentum* showed antifungal activity with the highest mean zone of inhibition ranged from (11.5±0.25 mm), lowest MIC (500 µg/ml) and MFC (1000µg/ml) against *Candida albicans A. niger*, and *A. flavus*.

The petroleum ether, chloroform, ethyl acetate acetone and methanol leaf extracts of *Oxystelma esculentum* showed a broad spectrum of antimicrobial activity against all the microorganisms tested. In the present study, Gram positive bacteria were more susceptible than Gram negative and fungal pathogens. The differences in the antimicrobial activity of crude extracts may be due to the amount of antimicrobial agent present in the extracts [11]. The selected plant belonging to Asclepiadaceae family were collected and their extract was tested for their antimicrobial activity against gram positive bacteria were found to be more susceptible than the gram negative bacteria. While the gram positive bacteria *Bacillus subtillis, Bacillus cereus* and *Staphylococcus aureus* was the most resistant and was inactive against the gram negative bacteria *Klebsiella pneumonia, Pseudomonas aureuginosa, Escherichia coli* and *Proteus vulgaris* [12].

Table 2. Antimicrobial activity of different extracts of *Oxystelma esculentum* Leaves

S. No.	Microbial strains/ solvents	Mean zone of inhibition[a] (mm)[b] Concentration of the extracts (µg/disc)				MIC (µg/mL)	MBC/ MFC (µg/mL)
		250	500	1000	Ciprofloxacin (5µg/disc)		
1	*Bacillus subtilis*						
	Petroleum ether	10.6±0.2	12.8±0.7	14.5±0.50	30.0±0.50	250	500
	Chloroform	10.8±0.35	13.0±0.40	15.5±0.25	29.5±0.50	125	250
	Ethyl acetate	12.5±0.36	14.0±0.40	16.5±0.36	29.0±0.50	62.5	125
	Acetone	10.8±0.50	12.7±0.28	14.6±0.40	28.0±0.50	125	250
	Methanol	16.7±0.36	18.8±0.40	20.5±0.50	29.5±0.50	31.25	62.5
2	*Bacillus cereus*						
	Petroleum ether	9.8±0.27	11.8±0.35	13.2±0.25	29.5±0.76	250	500
	Chloroform	10.5±0.25	11.2±0.35	13.5±0.25	29.0±0.76	125	250
	Ethyl acetate	10.2±0.30	12.5±0.25	14.8±0.38	28.5±0.78	125	250
	Acetone	11.0±0.25	12.0±0.50	14.0±0.50	28.0±0.25	125	250
	Methanol	15.8±0.40	17.0±0.25	19.2±0.11	29.0±0.50	62.5	125
3	*Staphylococcus aureus*						
	Petroleum ether	11.0±0.10	13.5±0.35	15.3±0.35	29.0±0.50	125	250
	Chloroform	12.0±0.36	13.8±0.37	15.8±0.30	28.6±0.55	62.5	125
	Ethyl acetate	13.3±0.25	16.8±0.3	19.7±0.35	29.1±0.55	31.25	62.5
	Acetone	11.5±0.50	14.0±0.50	17.8±0.25	29.5±0.50	62.5	125
	Methanol	18.5±0.20	21.8±0.3	23.5±0.25	29.6±0.82	15.62	31.25
4	*Klebsiella pneumonia*						
	Petroleum ether	8.7±0.45	10.9±0.35	13.0±0.28	29.0±0.50	250	500
	Chloroform	9.5±0.26	11.2±0.2	13.5±0.28	28.5±0.76	125	250
	Ethyl acetate	10.5±0.50	12.7±0.25	14.5±0.50	30.0±0.50	125	250
	Acetone	9.0±0.25	10.8±0.28	13.5±0.57	29.5±0.50	250	500
	Methanol	14.5±0.25	16.7±0.40	18.8±0.25	29.8±0.65	62.5	125
5	*Pseudomonas aeruginosa*						
	Petroleum ether	8.0±0.25	10.3±0.28	12.5±0.27	28.1±1.00	250	500
	Chloroform	8.6±0.25	10.5±0.25	12.5±0.36	30.0±0.32	125	250
	Ethyl acetate	10.8±0.36	12.0±0.25	14.0±0.50	29.5±0.50	125	250
	Acetone	8.8±0.56	10.5±0.15	12.5±0.50	29.0±0.28	250	500
	Methanol	13.5±0.28	14.2±0.30	16.7±0.35	27.5±0.50	62.5	125

S. No.	Microbial strain	Mean zone of inhibition[a] (mm)[b] Concentration of the extracts (µg/disc)				MIC (µg/mL)	MBC/ MFC (µg/mL)
		250	500	1000	Amphotercin- B (100units/disc)		
6	Escherichia coli						
	Petroleum ether	7.8±0.26	9.5±0.28	11.8±0.35	29.1±0.28	500	1000
	Chloroform	8.5±0.28	10.3±0.25	12.0±0.50	29.5±0.57	125	250
	Ethyl acetate	8.5±0.50	10.5±0.25	12.7±0.41	28.0±0.50	250	500
	Acetone	8.0±0.50	10.2±0.28	12.0±0.20	27.5±0.50	250	500
	Methanol	10.5±0.43	12.7±0.25	14.8±0.25	26.0±0.56	125	250
7	*Proteus vulgaris*						
	Petroleum ether	7.5±0.50	8.8±0.30	10.5±0.25	26.5±0.15	500	1000
	Chloroform	7.8±0.28	9.5±0.20	12.2±0.35	26.8±0.76	500	1000
	Ethyl acetate	7.8±0.56	9.6±0.57	11.8±0.50	28.0±0.50	250	500
	Acetone	8.5±0.25	11.7±0.2	13.0±0.25	27.5±0.50	250	500
	Methanol	8.5±0.50	10.7±0.25	13.7±0.25	26.5±0.50	250	500
8	*Candida albicans*						
	Petroleum ether	7.5±0.50	8.0±0.28	11.5±0.25	16.9±0.20	500	1000
	Chloroform	7.8±0.47	9.6±0.20	11.8±0.26	16.5±0.40	500	1000
	Ethyl acetate	7.3±0.28	8.3±0.40	10.5±0.45	16.8±0.65	500	1000
	Acetone	7.3±0.28	9.0±0.50	11.0±0.50	16.1±0.57	500	1000
	Methanol	7.3±0.50	8.5±0.26	10.5±0.50	16.5±0.50	500	1000
9	*Aspergillus niger*						
	Petroleum ether	8.7±0.45	10.9±0.35	13.0±0.28	29.0±0.50	250	500
	Chloroform	7.3±0.57	8.2±0.25	10.5±0.30	19.0±0.76	500	1000
	Ethyl acetate	7.5±0.50	8.5±0.30	10.8±0.25	19.5±0.76	500	1000
	Acetone	8.2±0.25	10.8±0.20	12.0±0.25	19.6±0.76	250	500
	Methanol	7.5±0.50	9.5±0.3	12.0±0.30	18.5±0.76	250	500
10	*Aspergillus flavus*						
	Petroleum ether	7.1±0.57	8.0±0.50	10.0±0.50	19.0±0.16	500	1000
	Chloroform	7.2±0.25	9.0±0.15	11.0±0.50	19.0±0.40	500	1000
	Ethyl acetate	7.5±0.50	8.6±0.40	11.2±0.35	19.0±0.11	500	1000
	Acetone	7.2±0.50	8.2±0.50	10.8±0.36	18.0±0.25	500	1000
	Methanol	8.5±0.25	10.5±0.26	12.8±0.28	18.0±0.50	250	500

The evaluation of antimicrobial potential by disc diffusion method indicates that all the bacterial tested organisms showed growth inhibition towards the plant extract, with differing sensitivity. Among the bacterial pathogens, *S. aureus* is more sensitive when compared to other bacteria. Gram-positive bacteria were exhibited more sensitive to

plant extracts when compared to Gram-negative bacteria [13]. The disc diffusion bioassay showed that the methanol leaf and stem extracts have the higher activity against all gram positive bacteria than the gram negative bacteria and antifungal activity of different concentration viz., 250 µg/ml, 500 µg/ml and 1000 µg/ml to assess the antifungal activity against various fungal strains. Among all the fungal isolates tested interestingly all the isolates exhibited maximum percentage growth inhibition at 1000 µg/ml concentration [14]. In addition, these results confirmed the evidence in previous studies reported that the methanol is a better solvent for more consistent extraction of antimicrobial substances from medicinal plant compared to other solvents, such as methanol, ethyl acetate and acetone, [15, 16, 17, 18].

CONCLUSION

It is concluded that methanol leaf extract of *Oxystelma esculentum* had a potential antimicrobial activity against all the microorganisms tested. Based on this study, isolation and identification of antimicrobial compound from methanol extract of *Oxystelma esculentum* will fetch a new natural antimicrobial agent.

Acknowledgement
The authors are thankful to Dr. K. Arumugam, Professor and Head, Department of Botany, Annamalai University for providing necessary laboratory facilities.

REFERENCES

[1] S.A Dahanukar, R.A Kulkarni, N.N Rege. *Indian Journal of Pharmacology*, **2000**, 32, S81–S118.
[2] I. Ahmad, Z. Mehmood, F. Mohammad. *J. Ethanopharmacol*, **1998**, 62: 183-193..
[3] S. Ashish, Q. Sadaf, S. Kavitha, A.K. Noor. *J. Exp. Sci*, **2011**, 2(10): 50-54.
[4] EK. Barbour, MA. Shanf, VK. Sagherian, AN. Habre, RS. Talhouk, SN. Talhouk. *J Ethnopharmacol*, **2004,** 93: 1-7.
[5] AW. Baur, WMM. Kirby, JC. Scherris, M. Turck. *Am j Clin pathol*, **1966,** 45:493-496.
[6] JD. Britto, SR. Sebastian, *Int J Pharm Sci*. **2012**, 5, 257- 259.
[7] L.M. Dhar, M.M. Dhar, B.N. Dhawan, B.N. Mehrotra, C. Ray. *Indian Journal of Experimental Biology*, **1968**, 6, 232–247.
[8] D.A. Dias, S. Urban, U. Roessner, *Metabolites*, **2012,** 2 303–336.
[9] JN. Eloff, *J. Ethnopharmacol*, **1998,** 60: 1-8.
[10] HM. Ericsson, JC. Sherris. *Acta Pathol Microbiol Scand*, **1971,** 217: 1-90.
[11] IB. Harborne, Phytochemical methods. Aguide to modern techniques of plant analysis. 2nd Edition, chapman and Hall, new York, **1973,** 88-185.
[12] B. Jayaprasad, P.S. Sharavanan. *RJPBCS*, **2015,** 6(1):1416- 1421 (2015).
[13] T. Karting, F. Still, F. Reinthaler. *J Ethnopharmacol*, **1991,** 135: 155-157.
[14] J. Lin, AR. Opoku, MG. Keller, AD. Hutching, SE. Terblanche, AK. Jager, JV. Staden. *J. Ethanopharmacol*, **1999,** 68: 267-274.
[15] Z.C. Maiyo, R.M. Ngure, J.C. Matasyo, *et al*., Afr. *J. Biotechnol.* **2010.** (9) 3178–3182.
[16] A. Mirzaeil, MK. Toori, N. Mirzaei, RG. Shirazi. *L. Sci. J*, **2013,** 10(7s): 108 –1091.
[17] R. Perumal Samy, S. Ignacimuthu. *Journal of Ethnopharmacology* **1998,** 62, 173–178.
[18] R. Perumal Samy, S. Ignacimuthu, *Journal of Ethnopharmacology* **2000,** 69, 63–71.
[19] JCA. Tanaka, CC. Da Silva, AJB. De Oliveira, CV. Nakamura, BP. Dias Filho. Braz. *J. Med. Bio. Res*, **2006,** 39: 387-391.
[20] G.E. Trease, W.C. Evans, harmacognosy 2nd Eoln. Braille Tiridel and Macmillan publishers. **1989,** Pp.242-245.
[21] J. Vaijayanthimala, NR. Prasad, C. Anandi, KV. Pugalendi. *Journal of Natural Remedies*, **2004,** 4(1): 26 – 31, (**2004**).
[22] A. Bauer, WWMM. Kirby, JC. Scherris, M. Turck, *J, Clin. Pathol.*, **1966,** 45,493-496.