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Anti microbial activity of Methanol Extract of *Oxystelma Esculentum*

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Abstract:

Bacterial and fungal infections were some of the most serious global health issues of the present century. Numerous biologically active plants have been discovered by evaluation of ethnopharmacological data and these plants may offer the local population immediately accessible therapeutic products. Several herbs were known to possess medicinal value including anti-microbial properties. *Oxystelma esculentum* is used medicinally in Egypt. Different parts of these plants have been claimed to be effective in a wide spectrum of diseases. The study was based on the effect of herbal medicine or natural products on candidiasis which is a fungal disease caused by the fungi *Candida albicans*. The present investigations were, therefore, proposed in evaluate the efficacy of the methanol extract of *Oxystelma esculentum* (MEOE). The antibacterial studies confirmed that MEOE had zone of inhibition, but the MIC of MEOE is 500µg/ml in two fold serial dilution method ensuring no prominent action on the tested bacterial strains.The antifungal studies confirmed that MEOE had a effective zone of inhibition against *C. albicans* and *C. neoformans*. In MIC studies, the MEOE had more effect on *C. albicans*, thus giving a lead for further in vivo anticandidal studies.

Key words: *Anti-microbial, Herbs, Methanol extract*

Introduction:

Bacterial and fungal infections were some of the most serious global health issues of the present century. Numerous biologically active plants have been discovered by evaluation of ethnopharmacological data, and these plants may offer the local population immediately accessible therapeutic products[1].

Several herbs were known to possess medicinal value including anti-microbial properties[2]. The extensive use of synthetic drugs, excessive unwanted medication will cause increasing side effects in the body, sometimes, the toxic effects produced by the administration of drugs is much more a serious problem than that of the disease itself. These factors compelled to us to search for safe formulation from alternative medical system. It is believed that alternative medical systems were in general devoid of side effects in the body. As far as allopathic drugs were concerned they seem to target directly against the disease causing organisms whereas, medicines of alternative system appear to strengthen body's defence mechanism first which inturn will tackle the diseases[3].

Oxystelma esculentum is used medicinally in Egypt. Different parts of these plants have been claimed to be effective in a wide spectrum of diseases. The present work is based on the effect of herbal medicine or natural products on candidiasis which is a fungal disease caused by the fungi *Candida albicans*.

In our present study the literature review reveals that there is no pharmacological work on antifungal activity has been carried out in the plant *Oxystelma esculentum.* The present investigations were, therefore, proposed in evaluate the efficacy of the methanol extract of *Oxystelma esculentum* (MEOE) against anti fungal microbes.

Materials and Methods:

Collection, plant authentication and pharmacognostical study

The pharmacognostic studies were designed so as to bring out the differences among the species of the same family in anatomical aspects, both in entire and powdered forms. The studies include a detailed anatomy and analytical parameters of leaf of *Oxystelma esculentum*.

Preparation of methanolic extract of oxystelma esculentum

The collected leafs were air dried, pulverized in to a coarse powder and sieved. The dried powdered material was defatted with petroleum ether (60-80^{o}C) and was further extracted with methanol in a soxhlet apparatus. The extract was filtered and the solvent was removed by distillation under vaccum. The dried

MEOE was suspended in distilled water and used for further studies. This residue subjected to Thin Layer Chromatography. Analytical Thin Layer Chromatography was performed on precoated sheets of silica gel G of 0.25 mm thickness containing PF 254 indicator.

Antibacterial Activity

Methanolic extract of *Oxystelma Esculentum* were dissolved in saline to get a stock solution of 1000 μg/ml to 3.625μg/ml concentration and were used for the further in vitro studies

Bacterial Strains

The various organisms used in the present study include *Staphylococcus aureus, Baceillus subtillis, Escherichia Coli, B. coagulans, B.mageterium, P.aeruginosa ,S.typhi, Shigella* were collected from National collection of industrial microorganisms (NCIM), National Chemical Laboratory, Pune, Maharastra, India. These organisms were maintained on nutrient agar slopes and the organisms were confirmed by biochemical test.

Preparation of standard bacterial suspensions

The average number of viable *Bacillus subtilis,Escherichia coli, Staphylococcus aureus,B. coagulans,B.mageterium,P.aeruginosa, S.typhi, Shigella* organisms per ml of the stock suspensions was determined by means of the surface viable counting technique[4]. About (10^8-10^9) colony-forming units per ml was used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

Preparations of Media

Muller Hinton Agar (MH, Hi media) was used. The formula (gm/litre) Beef 2g, casein acid hydrolysate 17.5g, starch 1.5 g and agar 17g; pH 7.4 ± 0.2.

About 38g of MH agar was weighed and dissolved in 1000 ml of distilled water and adjusted to pH 7.3 ± 0.2, sterilized by autoclaving at 121^{o}C for 15 minutes at 15 psi pressure and was used for sensitivity tests.

Cup-Plate Method[5]

The cup-plate agar diffusion method was adopted to assess the antibacterial activity of the prepared extracts. 0.6 ml of standardized bacterial stock suspensions (10^8-10^9) colony – forming units per ml was thoroughly mixed with 60 ml of sterile nutrient agar. 20 ml of the inoculated nutrient agar were distributed into sterile Petri dishes. The agar was left to set and in each of these plates 4 cups, 10mm in diameter, were cut using a sterile cork borer No.4 and the agar discs were removed. Cups were filled with 0.1ml of extracts, Gentamycin and saline using microliter-pipette and allowed to diffuse at room temperature for two hours. The plates were the incubated in the upright position at 37^{o}C for 18 hours. After incubation the diameter of the results and growth inhibition zones were measured, averaged and the mean values were recorded.

Two fold dilution method

The turbidimetric assay of drug potency is based on inhibition of microbial growth as indicated by measurement of the turbidity (transmittance) of a suspension of suitable microorganisms in a fluid medium to which have been added graded amounts of the test compounds and known concentration of reference material. The MIC is the minimum concentration at which the extract inhibits the growth of the microorganism compwere with Gentamycine as a control.

Anti-Fungal Activity

Preparation of Media[6]

Sabourand Dextrose Agar (Hi-media) media (SDA) was used for cultivation of fungi and particularly pathogenic fungi associated with skin infections[6]. Formula gm/litre Peptone 10g,dextrose 40g and agar 15g; pH5.6 ± 0.2. 65 gm of SDA was dissolved in 1000ml of distilled water. The medium was sterilized by autoclaving at 121^{o}C for 15 minutes at 15psi pressure.

Preparation of the extracts

The plant extracts namely methanolic extract of *Oxystelma Esculentum* were dissolved in saline to get a stock solution of 1000 µg/ml concentration and were used for the *in vitro* studies.

[7]The fungi were maintained on SDA slopes of *C. albicans, C. neoformans T. rubrum S. cerevisiae, A. niger, A. flavus R. arrhizus* fugai.

Cup-plate method

The cup-plate agar diffusion method to assess the antifungal activity of the prepared extracts. 0.6 ml of standardized fungal stock suspensions (10^8-10^9) colony – forming units per ml was thoroughly mixed with 60 ml of sterile nutrient agar. 20 ml of the inoculated nutrient agar were distributed into sterile Petri dishes[8,9]. The agar was left to set and in each of these plates 4 cups, 10mm in diameter, were cut using a sterile cork borer No.4 and the agar discs were removed. Cups were filled with 0.1ml of extracts, Ketaconazole and saline using microliter-pipette and allowed to diffuse at room temperature for two hours. The plates were the incubated in the upright position at 37^{o}C for 18 hours[10]. After incubation the diameter of the results and growth inhibition zones were measured, averaged and the mean values were recorded.

Two fold dilution method

[11]The turbidimetric assay of drug potency is based on inhibition of microbial growth as indicated by measurement of the turbidity (transmittance) of a suspension of a suitable microorganisms in a fluid medium to which have been added graded amounts of the test compounds and known concentration of reference material[12]. The MIC is the minimum concentration at which the extracts inhibits the growth of the microorganism.

Results and Discussion:

***In vitro* anti-fungal and antibacterial studies**

Doses of MEOE ranging from 1000µg/ml to 3.625µg /ml of the broth were tested for antibacterial activity against eight strains of both Gram positive and Gram negative bacteria, 24 h cultures of containing 1 x106 cells/ml, using two fold serial dilution and the cup plate method. The results reveal that MEOE has a zone of inhibition at 25 mm, 21 mm, and 20 mm for *S. aureus, B. subtilis* and *E.coli*, respectively in cup plate method. An MIC at 500µg/ml in two fold serial dilution method was observed for these bacterial strains (Table No.1). There was no prominent action on the other tested bacterial strains

The plant extracts were tested for anti-fungal activity against seven different fungal organisms. The results were recorded in Tables 2 and 3. The results reveal that MEOE possess zone of inhibition 25 mm against *C. albicans*, and 20 mm against *C. neoformans*. An MIC at 12.5µg/ml against *C. albicans* and 50 µg/ml against *C. neoformans*, and 500µg/ml against other tested fungal organisms were observed. MEOE possess maximum efficacy against *C. albicans*, thus giving a lead for further in vivo anticandidal studies. The results were comparable with ketoconozole, which show an MIC at 6.25µg/ml against all tested fungal organisms.

Conclusion:

The antibacterial studies confirmed that MEOE had zone of inhibition, but the MIC of MEOE is 500µg/ml in two fold serial dilution method ensuring no prominent action on the tested bacterial strains. The antifungal studies confirmed that MEOE had a effective zone of inhibition against *C. albicans* and *C. neoformans*. In MIC studies, the MEOE had more effect on *C. albicans*, thus giving a lead for further *in vivo* anticandidal studies. In future we can formulate the active constituent into a topical dosage form such as Gels, Creams, Ointments. *In vitro* evaluation of the dosage form and further stability studies, In vivo evaluation for confirming its wound healing properties and small scale

Table 1: Effect of the extracts on selected bacterial strains (Cup plate method &two fold dilution method)

Sl. No.	Organisms	Anti Bacterial (Cup Plate Method)			Two fold Dilution Method	
		Zone of Inhibition (mm)			Minimum Inhibitory Concentration(MIC) (µg/ml)	
		Concen-tration of Extract	MEOE	Standar d Drug	Concen-tration of Extract	Standard Drug
1.	*S.aures(+)*	**1000 25**		**32**	**500**	6.25
2.	*B.coagulans(+)*	**1000 16**		**31**	**1000**	6.25
3.	*B.Subtilis(+)*	**1000 21**		**32**	**500**	6.25
4.	*B.mageterium(+)*	**1000**	19	28	**1000**	3.625
5.	*E.Coli(-)*	**1000 20**		**34**	**500**	6.25
6.	*P.Aeruginosa*	**1000 15**		**25**	**1000**	6.25
7.	*S.Tiphi(-)*	**1000 16**		**32**	**1000**	3.625
8.	*Shigella(-)*	**1000 16**		**25**	**1000**	6.25

Strenth of Inoculumns:1×10^{7} cells/ml(24^{th} Culture) Drugs: Gentamycin, MEOE (1mg/ml stock Solution:0µg/disc) (+) Gram-Positive Bacteria, (-) Gram-Negative Bacteria.

Table 2: Effect of the extracts on selected fungal strains (Cup and plate method)

Sl.No.	Organisms	Concentration of the extract (µg/ml)	Zone of inhibition (mm)	
			MEOE	Standard Drug
1.	***C. albicans***	1000	25	28
2.	***C. neoformans***	1000	20	29
3.	***T. rubrum***	1000	14	22
4.	***S. cerevisiae***	1000	16	26
5.	***A. niger***	1000	15	26
6.	***A. flavus***	1000	19	32
7.	***R. arrhizus***	1000	16	34

Strength of inoculumns: $1x10^{7}$ cells / ml (48h culture) Drugs: Ketoconozole, MEOE (1 mg/ml stock solution; 10 µg/disc).

Table 3: Effect of the extracts on selected fungal strains (Two fold serial dilution method)

Sl.No.	Organisms	Minimum Inhibitary Concentration	
		MEOE Standard	Drug
1.	***C. albicans***	12.5	6.25
2.	***C. neoformans***	50	6.25
3.	***T. rubrum***	500	6.25
4.	***S. cerevisiae***	500	6.25
5.	***A. niger***	500	6.25
6.	***A. flavus***	500	6.25
7.	***R. arrhizus***	500	6.25

Strength of inoculumns: $1x10^7$ cells / ml (48h culture) Drugs: Ketoconozole, MEOE (1 mg/ml)

clinical trials were the scope behind this studies.

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