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Antimicrobial activity of *Mimusops elengi* Linn. unripe fruit extracts

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ABSTRACT

In this study, *Mimusops elengi* Linn. unripe fruits extracts were tested for their In vitro antimicrobial activities. The antimicrobial property of *Mimusops elengi* Linn. plant extracts have been carried out by disc diffusion susceptibility method. Different types of microbial strains have been used for the estimation of antimicrobial effect of *Mimusops elengi* Linn. unripe fruits extracts. The pet ether, benzene, chloroform and methanol extracts of unripe fruits *Mimusops elengi* Linn. gave the varying values of inhibition zone diameter, on their application against the microorganisms with the safe conclusion on the fact that the solvents could extract the different bio-organics varying in number and antimicrobial potential(s).
The concentration increase of the extracts resulted in the increase of inhibition zone diameter values resulting in the increase in the antimicrobial activities of the extracts. Among all the plant extracts, the methanol extracts was found to bear the highest antimicrobial potential against the microbes, followed by the chloroform extracts of the plant.
The methanol extracts were found to possess the antimicrobial potential in between ethyl acetate extracts and the water extracts. The water extracts were evaluated to have little effect upon the growth of microorganisms.

Keywords: Antimicrobial; Mimusops elengi Linn.*; extracts*, microbial strains, MIC

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INTRODUCTION

Mimusops elengi Linn. tree is the native of western peninsula. The tree is found in south India in dry evergreen forests from the Krishna southwards and in ravines in the hills up to 20 meter along western coast and lower ghats in moist evergreen forests. The fruits are sweet and sour, aphrodisiac, diuretic, astringent to the bowels, good in gonorrhoea. The ripe fruit pulp is sweetish and astringent and has been used in treating chronic dysentery **[1].**

In recent years, human pathogenic microorganisms have grown resistance in antiphon to the indiscriminate utilization of commercial antimicrobial drugs frequently employed in the treatment of infectious diseases. This situation, the inadmissible side effect of certain antibiotics, and the emergence of previously uncommon infections, has forced scientists to look for new antimicrobial substances from various sources, such as medicinal plants **[2, 3].** The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new anti-infective agents **[4, 5]**

MATERIALS AND METHODS

Plant material: Fresh unripe fruits of *Mimusops elengi* Linn. were collected from Salipur, Cuttack, Odisha, India. The plant parts was authenticated by Dr. Gita Rath, Department of Botany, Salipur Autonomous College, Salipur and given the voucher specimen no. 001/18. The air dried powdered fruits was loaded into soxhlet apparatus and was subjected to extraction for about 72 hours with petroleum ether (60-80ºC), benzene, chloroform and methanol successively. After extraction the solvent was distilled off and the extract was concentrated under reduced pressure using rotary evaporator. The extracts were stored in a closed bottle and kept in refrigerator until tested.

The *in vitro* antimicrobial activity: The pet ether, benzene, chloroform methanol of plant were evaluated for activity against medically important bacteria such as Staphylococcus *Escherichia hermanii* (MTCC-9144), *Staphylococcus pyogenes* (MTCC-442), *Staphylococcus aureus* (MTCC-9886), *Pseudomonas aeruginosa* (MTCC-10070), *Bacillus subtilis* (MTCC-441) and *Bacillus cerus* (MTCC-1305); and fungi *Aspergillus niger* (MTCC-282), *Aspergillus flavus* (MTCC-7589), *Candida albicans* (MTCC-183), *Fusarium oxyporum* (MTCC-4818), *Fusarium solani* (MTCC-350) and *Trichophyton mentagrophytes* (MTCC-9533).The invitro antimicrobial activity was performed disc diffusion method. The ethanolic and aqueous extracts showed minimum antimicrobial activity when compared to methanolic extracts. The methanolic extract *Mimusops elengi* Linn. raw fruits extract showed the maximum activity against Staphylococcus sp. The applicability of plant extracts with known antimicrobial claim can be of great connotation in therapeutic treatments.

Microbial cultures: The following microorganisms were used to test the antimicrobial activity of the extracts. Pure isolates of bacteria *Escherichia hermanii* (MTCC-9144), *Staphylococcus pyogenes* (MTCC-442), *Staphylococcus aureus* (MTCC-9886), *Pseudomonas aeruginosa* (MTCC-10070), *Bacillus subtilis* (MTCC-441) and *Bacillus cerus* (MTCC-1305); and fungi *Aspergillus niger* (MTCC-282), *Aspergillus flavus* (MTCC-7589), *Candida albicans* (MTCC-183), *Fusarium oxyporum* (MTCC-4818), *Fusarium solani* (MTCC-350) and *Trichophyton mentagrophytes* (ATCC-9533) were collected.

Medium preparation: 62.5 gm dehydrated culture medium was added to 1 liter of distilled water. It was heated with repeated stirring and boiled for one minute to dissolve the ingredients completely. The prepared media was distributed and autoclaved at 115^{o}C for 30 minutes. Final pH of the medium was maintained at 5.6 ± 0.2 by adding 0.1 N hydrochloric acid.

Inoculum preparation: Nutrient broth and Sabouraud dextrose agar (SDA) were used for growing and diluting the microorganism suspensions. Inoculum from bacterial cultures were prepared by picking colonies from 24 h old cultures. Colonies were suspended in 5 ml of a solution containing 0.145 mol of saline per liter. The density was adjusted by spectrophotometer to that of a 0.5 McFarland standard at a wavelength of 530 nm to yield a stock suspension of 1 x 10^6 to 5 x 10^6 cells per ml. Two different inoculum sizes were evaluated: 1 x 10^6 to 5 x 10^6 cells per ml and a 1:100 dilution containing 1 x 10^4 to 5 x 10^4 cells per ml. Plates were swabbed or sponged in three directions.

Fungal cultures were aseptically inoculated on petri dishes containing autoclaved, cooled, and settled SDA medium. The petri dishes were incubated at 31^{0}C for 48 h to give white round colonies against a yellowish background. These were aseptically subcultured on SDA slants. The yeast colonies from SDA slants were suspended in sterilized 0.9% sodium chloride solution (normal saline), which was compared with McFarland solution. According to the manufacturer's directions, 1 ml of yeast suspension in normal saline was added to 74 ml of sterile medium and kept at 45°C to give a concentration of 2 X 107 cells/ml.

Disc diffusion susceptibility method: When a filter paper disc impregnated with a chemical is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a "zone of inhibition" **[6].**

Disc diffusion assay: Stock solutions of different extracts of *Mimusops elengi* Linn. were prepared separately by initially dissolving 0.5 gm of the extract in 0.5 ml of DMSO to obtain a stock solution of concentration 1000 mg/ml. From this stock solution, concentrations of 10 mg/ml were prepared by serial dilution. From the above stock solution further dilutions were made to get 10, 20, 30, 40, 60 µg/ml working solutions. The Kirby-Bauer diffusion method was used in the antimicrobial screening. Nutrient agar was inoculated with a microbial cell suspension (200 µl in 20 ml of medium) and poured into sterile petri dishes followed by cross-streaking with the same wire loop to achieve uniform spread on the plate. Sterile filter paper discs 6 mm in diameter were impregnated with 20 µl of each extract concentration (30 and 60 µg/ml), which were prepared using the same solvents employed to dissolve the plant extracts, and placed on the inoculated agar surface. Standard 6-mm discs containing rifampicin 30 µg/disc was used as positive control. Negative controls were made using paper discs loaded with 20 µl of the solvents. After pre-incubation for 2 h in a refrigerator the plates were incubated overnight at 37 °C for 18-24 h. In contrast, fungal cultures were incubated at 31 °C for 48 h in SDA medium using miconazole 10 µg/disc **[7].** Negative controls were made using paper discs loaded with 20 µl of the solvents. At the end of the incubation period antimicrobial activity was evaluated by measuring the zones of inhibition.

Determination of the minimum inhibitory concentration (MIC): Double strength nutrient agar was prepared by dissolving 28 gm in 500 ml of distilled water which was swirled and mixed thoroughly by heating to allow uniform dissolution after which 5 ml of it was dispensed into universal bottles and sterilized in an autoclave at 121^{o}C for 15 min. The agar was allowed to cool to 45^{o}C and each graded solution was then mixed gently with molten double strength nutrient agar in a petri dish and allowed to solidify for one hour. Extracts' concentrations of 100, 90, 80, 70, 60, 50, 40, 30, 20, 10 and 5 µg/ml respectively were prepared by serial dilution. Each plate was divided into six equal sections and labeled accordingly to correspond to six test organisms. Two 6 mm diameter paper discs (Whatman No.1) were placed aseptically into each labelled section of the plate using sterilized forceps. With an automatic micropipette, 20 µl of each bacterial suspension was taken and transferred aseptically and carefully into each appropriate pre-labeled paper disc on the agar plates. The plates were incubated for 24 h at 37^{o}C after which they were observed for growths or death of the test organisms. In contrast, fungal cultures were incubated at 31 °C for 48 h in SDA medium. The lowest concentration inhibiting growth was taken as the minimum inhibitory concentration (MIC). The average of 3 values was calculated and that was the MIC for the test material.

RESULTS

The present study was conducted to investigate the antimicrobial activity of *Mimusops elengi* Linn. extracts against some strains of bacteria and fungi. The antimicrobial results or zone of inhibition obtained using the disc diffusion method is sited in **(Table 9)**. The methanol extract of *Mimusops elengi* Linn. exhibited significant activity against all the tested bacteria and fungus. Chloroform extract showed moderate action against other tested microorganisms. The pet ether and benzene extracts of *Mimusops elengi* Linn. showed mild to no effect against the pathogens. The minimum inhibitory concentration of pet. ether, benzene, chlororform, and methanol extracts against pathogenic bacteria and fungi were recorded and shown **(Table 1, 2, 3, 4, 5, 6, 7 and 8).**

In the present study methanol extract of *Mimusops elengi* Linn. exhibited significant activity against all the tested bacteria and fungus. Methanol extract showed ≥ 16mm zone of inhibition at a concentration of 60µg/disc against *Escherichia hermanii, Staphylococcus pyogenes, Staphylococcus aureus, Bacillus subtilis , Bacillus cerus and* ≥ 11-15mm against *Psedomonas aeruginosa.* At a concentration of 30 µg/disc methanol extract showed ≥ 11-15mm zone of inhibition against *Escherichia hermanii, Staphylococcus pyogenes, Staphylococcus aureus, Bacillus subtilis and Bacillus cerus and* ≥5-10 mm zone of inhibition against *Pseudomonas aeruginosa.*

Chloroform extract showed ≥11-15mm zone of inhibition at a concentration of 60µg/disc against *Escherichia hermanii, Staphylococcus pyogenes, Staphylococcus aureus Bacillus subtilis , Bacillus cerus and Pseudomonas aeruginosa.* At a concentration of 30µg/disc chloroform extract

showed ≥5-10mm zone of inhibition against *Escherichia hermanii, Staphylococcus pyogenes, Pseudomonas aeruginosa, Bacillus cerus,* with the same concentration resulted in ≥11-15mm zone of inhibition against *Staphylococcus aureus and Bacillus subtilis.*

Mimusops elengi Linn. benzene extract showed ≥5-10mm zone of inhibition at a concentration of 60µg/disc against *Staphylococcus aureus and Pseudomonas aeruginosa.* Whereas zone of inhibition for other strains was found to be < 4mm. At a concentration 30µg/disc benzene extract showed < 4mm zone of inhibition against for all bacterial strain.

Mimusops elengi Linn. pet ether extract showed ≥5-10mm zone of inhibition at a concentration of 60µg/disc against *Staphylococcus aureus , Bacillus cerus* and <4mm against all other bacterial strains. At a concentration of 30µg/disc pet ether extract showed ≥5-10mm zone of inhibition against *Bacillus cerus* and the extract at same concentration exhibited <4mm against all other bacterial strains.

The methanol extract showed ≥16mm zone of inhibition at a concentration of 60µg/disc against the fungi *Aspergillus niger, Aspergilus flavus, Candida albicans, Trichophyton mentagrophytes* and ≥15mm zone of inhibition against *Fusarium oxyporum and Fusarium solani.* At a concentration of 30µg/disc methanol extract showed ≥11-15mm zone of inhibition against *Aspergillus niger, Aspergilus flavus, Candida albicans, Trichophyton mentagrophytes* and ≥6-10mm zone of inhibition against *Fusarium oxyporum and Fusarium solani.*
Mimusops elengi Linn. chloroform extract showed ≥16mm zone of inhibition at a concentration of 60µg/disc against *Candida albicans* , ≥11-15mm zone of inhibition against *Aspergillus niger, Aspergilus flavus, Fusarium oxyporum, Trichophyton mentagrophytes* and ≥6-10mm zone of inhibition against *Fusarium solani*. at a concentration of 30µg/disc chloroform extract showed ≥11-15mm zone of inhibition against *Candida albicans*, ≥6-10mm against *Aspergillus niger Fusarium oxyporum Trichophyton mentagrophytes* and 6mm zone of inhibition against *Aspergilus flavus, Fusarium solani and Candida albicans.*

Mimusops elengi Linn. benzene extract showed ≥6-10mm zone of inhibition against *Aspergillus niger Fusarium oxyporum Trichophyton mentagrophytes* and <4mm against *Aspergilus flavus Candida albicans, Fusarium oxyporum.* At a concentration of 30µg/disc benzene extract showed ≥6-10mm zone of inhibition against *Trichophyton mentagrophytes* and 6mm for all other fungi strains mentioned above. *Mimusops elengi* Linn. pet ether extract showed ≥6-10mm zone of inhibition against *Fusarium oxyporum, Trichophyton mentagrophytes* and 6mm against all other fungi strains. At a concentration of 30µg/disc pet ether extract showed 6mm zone of inhibition against all fungi strains.

All the extracts of *Mimusops elengi* Linn. showed moderate effects but methanol and chloroform extract showed significant activity . As the gram – ve bacteria are having more protective lipid layer than gram +ve bacteria therefore the antibacterial activity drug cannot diffuse easily through the protective lipid layer in gram –ve bacteria thereby showing less antibacterial activity. Whereas in gram +ve bacteria the antibacterial drug can diffuse easily inside the cell wall therefore showing increase antimicrobial activity. The zones of inhibition ranged from ≥6-18 mm for different extracts against bacteria and fungi. The methanol and chloroform extract had promising MIC values against all tested bacteria and fungi. The resistance of bacteria and fungi towards different drugs can be due to modification of the target site, bypass of pathways, decreased uptake (reduced intracellular concentration either of the antimicrobial agent, reducing membrane permeability or by active efflux pump), enzymatic inactivation or modification of the drug, or over production of the target.

In the present investigation, different extracts of Mimusops elengi Linn. extracts were evaluated for exploration of their antimicrobial activity against certain Gram negative and Gram positive bacteria, fungus which was regarded as human pathogenic microorganism.

DISCUSSION

Moreover it had long been documented that saponins, tannins and alkaloids are plants metabolites known for antimicrobial activity. Some of the distinguished compounds in these plants extracts may be culpable for the antibacterial activity observed and thus justifying their traditional use as medicinal plants for the treatment of bacterial gastroenteritis. It is important to execute the bio-autography of the extracts in order to evaluate and determine the exact antibacterial compound(s); unfortunately we were unable to perform this due to confined or limited facilities. The antimicrobial effects of *Mimusops elengi Linn*. may be attributed to various phytochemicals contained in its extracts, such as saponins, tannins and alkaloids.

CONCLUSION

The purpose of the study was to evaluate the antimicrobial activity of *Mimusops elengi* Linn.

extracts. The results of our experiments exhibited that different microbial strains exhibited different sensitivities towards the different extracts of *Mimusops elengi* Linn. At abreast, maximum pathogenic organisms are comely defiant to antibiotics. To conquer this alarming hitch, the discovery of novel active compounds against new targets is a matter of urgency. Utmost all of the medicinal plant extracted either in water or in organic solvents have biologically active compounds, which can be used in the synthesis of potent drugs. Thus, medicinal plants can provide protection largely against our natural pathogens.

On the basis of antimicrobial efficacy, methanol extract of *Mimusops elengi* Linn. was found to be effective than other extracts. Antimicrobial activity by disc diffusion assay exhibited broad spectrum of antimicrobial activity of methanol extract against bacteria and fungus. The intensity of antimicrobial action varied depending on the microorganism. Chloroform extract of *Mimusops elengi* Linn. exhibited appreciable antimicrobial activity. The pet ether and benzene extract of *Mimusops elengi* Linn. showed lesser antimicrobial activity. Further studies are neede to find and evaluate out the specific active compounds of the plant. We conclude that, it is possible to find best therapies for various infectious diseases from the plant extract.

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CONFLICTS OF INTEREST
There are no conflicts of interest.

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Table 1: MIC of *Mimusops elengi* Linn. pet ether extract on some pathogens (Bacteria).

Plant	Test organisms	Concentration (µg/ml) of *Mimusops elengi* Linn. pet ether extract										
		100	90	80	70	60	50	40	30	20	10	5
M. elengi	*Escherichia hermanii*	++	++	++	++	++	++	++	++	++	++	+++
	Staphylococcus pyogenes	-	-	-	-*	+	+	++	++	++	++	+++
	Staphylococcus aureus	-	-	-	-	-*	+	++	++	++	++	+++
	Pseudomonas aeruginosa	-	-	-*	+	+	++	++	++	++	++	++
	Bacillus subtilis	-	-	-*	+	+	++	++	++	++	+++	+++
	Bacillus cerus	-	-	-	-	-*	+	++	++	++	+++	+++

* = MIC concentration; - = No growth, + + = growth.

Table 2: MIC of *Mimusops elengi* Linn. benzene extract on some pathogens (Bacteria).

Plant	Test organisms	Concentration (µg/ml) of *Mimusops elengi* Linn. benzene extract										
		100	90	80	70	60	50	40	30	20	10	5
M.elengi	*Escherichia hermanii*	-	-	-*	+	++	++	++	++	++	+++	+++
	Staphylococcus pyogenes	-	-	-	-*	+	++	++	++	++	++	+++
	Staphylococcus aureus	-	-	-	-	-*	+	+	++	++	+	++
	Pseudomonas aeruginosa	-	-	-	-	-*	+	++	++	+++	+++	+++
	Bacillus subtilis	-	-	-	-*	+	+	+	++	++	+++	+++
	Bacillus cerus	-	-	-*	+	+	+	+	++	+++	+++	+++

* = MIC concentration; - = No growth, + + = growth.

Table 3: MIC of *Mimusops elengi* Linn. chloroform extract. on some pathogens (Bacteria).												
Plant	**Test organisms**	**Concentration (µg/ml) of *Mimusops elengi* Linn. chloroform extract.**										
		100	**90**	**80**	**70**	**60**	**50**	**40**	**30**	**20**	**10**	**5**
M.elengi	*Escherichia hermanii*	-	-	-	-	-	-	-	-*	+	++	+++
	Staphylococcus pyogenes	-	-	-	-	-	-	-	-*	+	++	++
	Staphylococcus aureus	-	-	-	-	-	-	-	-	-*	+	++
	Pseudomonas aeruginosa	-	-	-	-	-	-	-	-*	+	++	+++
	Bacillus subtilis	-	-	-	-	-	-	-	-	-*	+	+
	Bacillus cerus	-	-	-	-	-	-	-	-	-*	+	++

* = MIC concentration; - = No growth, + + = growth.

Table 4: MIC of *Mimusops elengi* Linn. methanol extract on some pathogens (Bacteria).												
Plant	**Test organisms**	**Concentration (µg/ml) of *Mimusops elengi* Linn. methanol extract**										
		100	**90**	**80**	**70**	**60**	**50**	**40**	**30**	**20**	**10**	**5**
M.elengi	*Escherichia hermanii*	-	-	-	-	-	-	-	-	-*	+	+
	Staphylococcus pyogenes	-	-	-	-	-	-	-	-	-*	+	++
	Staphylococcus aureus	-	-	-	-	-	-	-	-*	+	++	++
	Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-*	+	+
	Bacillus subtilis	-	-	-	-	-	-	-	-	-*	+	++
	Bacillus cerus	-	-	-	-	-	-	-	-	-*	+	++

* = MIC concentration; - = No growth, + + = growth.

Table 5: MIC of *Mimusops elengi* Linn. pet ether extract on some pathogens (Fungi).												
Plant	**Test organisms**	**Concentration (µg/ml) of *Mimusops elengi* Linn. pet ether extract**										
		100	**90**	**80**	**70**	**60**	**50**	**40**	**30**	**20**	**10**	**5**
M.elengi	*Aspergillus niger*	-	-	-*	++	++	++	++	++	++	+++	+++
	Aspergillus flavus	-	-	-	-*	+	++	++	++	+++	+++	+++
	Candida albicans	-	-	-*	+	++	++	+	++	++	+++	+++
	Fusarium oxyporum	-	-	-	-*	+	++	++	+++	+++	+++	+++
	Fusarium solani	-	-	-	-*	+	+	++	+	++	++	+++
	Trichophyton mentagrophytes	-	-	-	-	-	-*	+	++	++	+	++

* = MIC concentration; - = No growth, + + = growth.

Table 6: MIC of Mimusops elengi Linn. benzene extract on some pathogens (Fungi).												
Plant	**Test organisms**	**Concentration (µg/ml) of *Mimusops elengi* Linn. benzene extract**										
		100	**90**	**80**	**70**	**60**	**50**	**40**	**30**	**20**	**10**	**5**
M.elengi	*Aspergillus niger*	-	-	-	-	-	-	-	-	-*	+	++
	Aspergillus flavus	-	-	-	-	-	-	-	-*	+	++	++
	Candida albicans	-	-	-	-	-	-	-	-*	+	++	+++
	Fusarium oxyporum	-	-	-	-	-*	+	++	++	+++	+++	+++
	Fusarium solani	-	-	-	-	-*	+	++	++	+++	+++	+++
	Trichophyton mentagrophytes	-	-	-	-	-	-	-	-*	+	++	++

* = MIC concentration; - = No growth, + + = growth.

Table 7: MIC of *Mimusops elengi* Linn. chloroform extract on some pathogens (Fungi).											
Plant	**Test organisms**	**Concentration (µg/ml) of *Mimusops elengi* Linn. chloroform extract**									
		100	**90**	**80**	**70**	**60**	**50**	**40**	**30**	**20**	**10**	**5**
M.elengi	*Aspergillus niger*	-	-	-	-	-	-	-	-	-*	++	+++
	Aspergillus flavus	-	-	-	-	-	-	-	-*	+	++	+++
	Candida albicans	-	-	-	-	-	-	-	-	-*	+	++
	Fusarium oxyporum	-	-	-	-	-	-	-	-	-*	+	++
	Fusarium solani	-	-	-	-	-	-	-*	+	++	+++	+++
	Trichophyton mentagrophytes	-	-	-	-	-	-	-	-	-*	+	++

* = MIC concentration; - = No growth, + + = growth.

Table 8: MIC of *Mimusops elengi* Linn. methanol extract on some pathogens (Fungi).												
Plant	**Test organisms**	**Concentration (µg/ml) of *Mimusops elengi* Linn. methanolic extract**										
		100	**90**	**80**	**70**	**60**	**50**	**40**	**30**	**20**	**10**	**5**
M.elengi	*Aspergillus niger*	-	-	-	-	-	-	-	-	-*	+	++
	Aspergillus flavus	-	-	-	-	-	-	-	-	-*	+	++
	Candida albicans	-	-	-	-	-	-	-	-	-	-*	+
	Fusarium oxyporum	-	-	-	-	-	-	-	-	-*	+	++
	Fusarium solani	-	-	-	-	-	-	-	-	-*	+	++
	Trichophyton mentagrophytes	-	-	-	-	-	-	-	-	-	-*	+

. * = MIC concentration; - = No growth, + + = growth

Table 9: Antibacterial and antifungal activity of *Mimusops elengi* Linn. unripe fruit extracts											
Microorganisms	**Pet.ether (µg/disc)**		**Benzene µg/disc)**		**Chloroform µg/disc)**		**Methanol (µg/disc)**		**Rifampicin (µg/disc)**	**Miconazole (µg/disc)**	**DMSO**
	30	**60**	**30**	**60**	**30**	**60**	**30**	**60**	**30**	**10**	
Bacteria											
Escherichia hermanii	-	-	-	-	+	++	++	+++	+++	NT	-
Staphylococcus pyogenes	-	-	-	-	+	++	++	+++	+++	NT	-
Staphylococcus aureus	-	+	-	+	++	++	++	+++	+++	NT	-
Pseudomonas aeruginosa	-	-	-	+	+	++	+	++	+++	NT	-
Bacillus subtilis	-	-	-	-	++	++	++	++	+++	NT	-
Bacillus cerus	+	+	-	-	+	++	++	+++	++	NT	-
Fungi											
Aspergillus niger	-	-	-	+	+	++	++	+++	NT	+++	-
Aspergillus flavus	-	-	-	-	-	++	++	+++	NT	+++	-
Candida albicans	-	-	-	-	++	+++	++	+++	NT	+++	-
Fusarium oxyporum	-	+	-	-	+	++	+	++	NT	+++	-
Fusarium solani	-	-	-	+	-	+	+	++	NT	+++	-
Trichophyton mentagrophytes	-	+	+	+	+	++	++	+++	NT	+++	-

Experiments were done in triplicate. Disc diameter = 6mm.

Diameter of zone of inhibition: - = 6; + = 6-10; ++ = 11-15; +++ > 16; NT = not tested. DMSO had not shown any antimicrobial activity against the tested organisms.

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