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Isolation and Identification of therapeutic compounds from *Moringa oleifera* and its antimicrobial activity

Gopinath, L. R., Jeevitha, S., Gokiladevi, T., and Archaya. S.
Department of Biotechnology, Vivekanandha Educational Institutions, Namakkal, Tamilnadu, India.

Abstract: *Moringa oleifera, is commonly known as 'drumstick tree' or 'horseradish tree'. Every part of the plant has high nutritive value. In this study the leaf extracts of Moringa oleifera was prepared using 7 different solvents and phytochemically analysed in which methanol leaf extract showed presence of many compounds. Quantification of leaf extract showed presence of high amount tannins. Palmitic acid was isolated from the extract and antimicrobial activity of palmitic acid was studied at different concentration for both bacteria and fungi. The results revealed that though both Gram negative and positive bacterial species were inhibited the gram negative were resistant particularly P. aeruginosa and high zone of inhibition was observed with K. pneumonia. The plant has many pharmacological properties and need the scientific values from the researcher for commercialization*

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I. Introduction

Moringa oleifera is commonly known as drumstick and horse radish tree which belongs to the family *Moringaceae. M. oleifera* plant is native to sub-Himalayan tracts of Northern India. It ranges from tiny herbs to large trees which grow up to 5-10m height (Morton, 1991). The leaves, Flowers and immature pods from the Moringa tree were used as a nutritive vegetable in many parts of the country (Anwar and Banger, 2003; Anwar *et al*., 2005). Many papers reported that the leaves of *Moringa* was highly rich in Beta carotene, protein, vitamin C, Calcium, and potassium and it can also acts as an effective source of natural antioxidants (Dillard and German, 2000; Siddhuraju and Becker, 2003). Apart from being highly nutritive *Moringa* can also be treated as an important medicinal plant for treating many prolonged diseases including cancer. *Moringa* provides the rare combination of zeatin, quercetin, sistosterol, caffeoylquinic acid and kaempferol (Durgesh Kumar *et al*., 2013). More than 40 natural anti-oxidants with numerous other secondary metabolites of health importance are present in Moringa species (Khawaja Thahir *et al*., 2010). Different parts of the plant in *Moringa* is well known for its medicinal properties like antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antidiabetic, antioxidant, antifungal and antibacterial activities (Faroq Anwar *et al*., 2007). Among the various use of this plant species anti-cancer property is highly valued in the recent past by researchers though it is known from the time immemorial.

The leaves of *Moringa* contains bioactive compounds called β – sitosterol which are highly involved in the stabilization of the cholesterol level in the serum of the high fat diet fed rats (Ghasi *et al*., 2000) and it also shows the antiulcer effect which indicates that the antiulcer components were fully spread in this plant (Pal *et al*., 1995). *Moringa* leaves are highly rich in β – carotene and leutin which supplies the vitamin A that is highly responsible to prevent the night blindness and also the eye problems in the children. The juices of the *Moringa* leaves were also involved in the treatment of the conjunctivitis.

II. Materials and Methods

Collection and Identification of the Sample

Young leaves were collected from *Moringa oleifera* tree growing at Tiruchengode, Namakkal District and was identified and authenticated by Botanical Survey of India, Coimbatore.

Preparation of the Plant Extracts

Drying Process

The leaf of Moringa tree were collected, washed and dried under shade in room temperature for 15 days to avoid the loss of bioactive compounds present in it. The dried samples were grinded and stored in an air tight container for the further use.

Extraction Process

The powdered plant samples were dissolved in different solvents (Ethanol, Methanol, Acetone, Ethyl Acetate, Petroleum Ether, Water, Chloroform, Benzene) for 42 hrs at 75 rpm in orbital shaker. After the incubation the

extracts were filtered by using No.1 Whatman filter paper. The filtered extracts were stored in brown bottle to avoid the Phytochemical changes.

Phytochemical Screening of Secondary Metabolites

The Phytochemical screening for the identification of the secondary metabolites like Alkaloids, Flavonoids, Lignin, Tannins, Phenols, Terpenoids, Steroids, Glycosides, Saponins, Proteins and Carbohydrates were performed using the standard procedure.

Quantitative analysis of Secondary Metabolites

Quantitative analysis of Secondary Metabolites was done by using the standard procedure prescribed by Alkaloids (Harborne, 1973), flavonoids (Kumaran, *et al*., 2006), Saponins (Obadani, 2001), Terpenoids and tannins (Van-Burden and Robinson, *et al*., 1981) and phenolics (Mc. Donald, *et al*., 2001).

Extraction and Isolation palmitic acid

The crude leaf extract of *Moringa oleifera* was used to extract and isolate compound palmitic acid. The soluble portion from the crude methanol extract was prepared using n- hexane and then the filtrate was collected and again the filtrate was repeated with ethyl acetate, n- butanol and residue of methanol fraction was obtained at 600 ℃ in hot air sterilizing. In a Column, 50g of silica gel acts as a stationary phase, petroleum ether used has a mobile phase, followed by petroleum ether and ethyl acetate in the ratio of 9:1. The column was parked by wet parking method and allowed to keep for overnight with 3g of concentrated ethyl acetate fraction was dissolved in petroleum ether solution, soaked with cotton wool and placed on top of silica gel in the column. From the column fractions were collected in collection bottles. The column fraction's profiles were monitored by TLC to confirming the similarities of elutes based on the number and color of the spot

Antibacterial activity of *Moringa oleifera*

Antimicrobial activity of secondary metabolites were identified through bacteria such as *Vibrio cholerae, Salmonella typhi, Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Staphylococcus pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia* using disc diffusion method (Baurer *et al*., 1996). The cultures of bacteria were inoculated in broth media and grown at 37ºC for 18 hours. The Muller Hinton agar plates were prepared and each plate was inoculated with 18 hours old cultures (100µl) and spread evenly on the plate. The control plates were made with using different antibiotic disc. All the plates were incubated at 37ºC for 24 hours and the diameter of inhibition zone in mm were noted.

Antifungal Activity of *Moringa oleifera*

Antifungal activity of *Moringa oleifera* was carried out by the fungal pathogens such as *Candida albicans, Aspergillus fumigates,* and *Fusarium graminearum*. The stock cultures of fungus were revived by inoculating in potato dextrose agar media and grown at 37ºC for 72 hours. The potato dextrose agar plates were All the plates were incubated at 37ºC for 72 hours and the diameter of inhibition zone in mm were noted.

III. Results

Phytochemical analysis of *Moringa oleifera* was done for alkaloids, Saponins, proteins, flavonoids, Carbohydrates, Tannins, Phenols, Terpenoids, Steroids, Glycosides, and phytosteroids in leaf using different solvents like Ethanol, Methanol, Acetone, Ethyl Acetate, Petroleum Ether, Chloroform and Benzene and the results was tabulated (Table 1). Quantitative secondary metabolites estimated in the methanol leaf extract showed alkaloids of 3.29g/100gm, Flavanoids of 4.67 g/100gm, Lignins of 1.54 g/100gm, Tannins of 6.67 g/100gm, Phenols of 1.64 g/100gm, Terpenoids of 4.27 g/100gm and Saponins of 1.66 g/100gm (Table 2).

The antibacterial activities of the selected bacteria against palmitic acid in different concentration are as follows. Vibrio cholera showed high zone of inhibition 15.83mm in highest concentration of 15.0 µg/ml with p value < 0.0001 and f value 657.61. *Salmonella typhi* showed high zone of inhibition 13mm in highest concentration of 15.0 µg/ml with p value < 0.0001 and f value 255.48. *Escherichia coli* showed high zone of inhibition 12mm in highest concentration of 15.0 µg/ml with p value < 0.0001 and f value 202.46, *Bacillus subtilis* showed high zone of inhibition 17mm in highest concentration of 15.0 µg/ml with p value < 0.0001 and f value 545.83, *Staphylococcus aureus* showed high zone of inhibition 16.67mm in highest concentration of 15.0 µg/ml with p value < 0.0001 and f value 941.71, *Staphylococcal pneumonia* showed high zone of inhibition 18.50mm in highest concentration of 15.0 µg/ml with p value < 0.0001 and f value 255.48, *Pseudomonas aeruginosa* showed high zone of inhibition 10.83mm in highest concentration of 15.0 µg/ml with p value < 0.0001 and f value 410.91, *Proteus mirabilis* showed high zone of inhibition 11.50mm in highest concentration of 15.0 µg/ml with p value < 0.0001 and f value 300.45, *Klebsiella pneumonia* showed high zone of inhibition 20.50mm in highest concentration of 15.0 µg/ml with p value < 0.0001 and f value 833.70(Figure 1 and Table 3).

Antifungal activity of Palmitic acid isolated from *M. oleifera* showed high zone of inhibition against *Candida albicans* and least in *Fusarium graminearum* in all the concentration (Figure 2).

Antifungal activity of Palmitic Acid isolated from *M. oleifera* by using fungal species. *Candida albicans* showed high zone of inhibition (19.50mm) in high concentration of palmitic acid (15µg/ml) with p value <0.0001 and f value 441.62. *Aspergillus fumigates* showed high zone of inhibition (11.67mm) in high concentration of palmitic acid (15µg/ml) with p value <0.0001 and f value 397.48. *Fusarium graminearum* showed high zone of inhibition (10.50mm) in high concentration of palmitic acid (15µg/ml) with p value <0.0001 and f value 264.46 (Table 4).

IV. Discussion

Increasing side effects and resistant genes in the allopathic therapeutic chemical compounds increased interest on herbal medicines as a result of their minimum or nil side effects. These herbal medicines or bioactive compounds increasingly used in Ayurveda, unani, yoga, siddha, homeopathy and naturopathy. However, allopathic medicines are used for major diseases and ailments though 70% people still use non-allopathic system of medicine for many general ailments (Kiritikar and Basu, 1991). Only in the recent past herbal medicines are evaluated for specific diseases and infections like Cancer, AIDs, Hepatitis, etc.

In the present context screening of plants for active biochemical compounds is essential to evaluate their. Among the different herbal plant *M. oleifera* is widely used in India and elsewhere on the globe as common vegetable is rich in nutritional value and also fund to have variety of therapeutic properties. The root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil were used for variety of ailments in the indigenous system of medicine like inflammation, infectious diseases, cardiovascular problem, gastrointestinal problem, hematological problem, hepatorenal disorders etc., (Morimitsu *et al*., 2000).

Preliminary screening of *M. oleifera* with different solvents and different methods showed that Alkaloids were obtained in most of the solvents except chloroform and benzene. Saponins was absent in petroleum ether and Benzene as solvents. Flavonoids were absent in petroleum ether, chloroform and Benzene as solvents. However, all the compounds were present in the methanol extract. The difference in polarity of different solvents and their appropriate temperature their presence varies (Bhat *et al*., 2005; Heinrich *et al*., 2005; Kalia, 2005). Presence or absence of different compounds are also varies from study to study where Abalaka *et al*., (2012) showed absence of tannins in chloroform extract of M. oleifera where as Bukar *et al*., (2010) showed the presence with chloroform as solvent.

Quantitative analysis of secondary metabolites of *M. oleifera* showed appreciable amount of tannins, flavanoids, alkaloids and terpenoids. Ojialko, (2014) observed by similar higher amount of tannins and saponins where as flavanoids and alkaloids were extremely low in acetone, N-hexane and ethyl acetate.

Terpenoids are the largest group of compounds of plant secondary metabolites used as flavours and fragrance in foods and cosmetics. Apart from their use as flavours and fragrance they also have medicinal property like anti-carcinogenic (e.g. Taxol and perillyl alcohol) antimalarial (e.g. Artemisinin) anti-ulcer, antimicrobial or diuretic (e.g. glycyrrhizin) (Bertea, *et al*., 2005). However, reports also reported alkaloid is one of the largest phytochemical compound where even a powerful pain killer drug was developed (Kam and Liew, 2002). It was also reported that these compounds have toxicity against cells of foreign organisms which is reported as anticancer property (Nobori *et al*., 1994). The derivatives of alkaloids were also reported to have analgesic, antispasmodic and antibacterial effects (Okwu and Okwu, 2004). Flavonoids, of *Moringa oleifera* also exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, antiallergic, cytostatic and antioxidant properties (Hodek *et al*., 2002). Flavanoids also prevent oxidative cell damage with strong anticancer activity and have water-soluble antioxidants and free radical scavengers (Stauth, 2007) and they also lower the heart disease. Saponin has potential to precipitate and coagulate the red blood cells and form foams in water, showing haemolytic activity, binds with cholesterol with bitterness prevents excessive intestinal absorption of cholesterol and reduces cardiovascular diseases (Okwu, 2004; Sodipo *et al*., 2000; Akinpelu and Onakoya, 2000). It is also reported that total saponins have antioxidant, antimutagenic and anticancer property (Luo *et al*., 2002).

M. oleifera leaf extract was reported to have varying degree of antibacterial activity against different bacterial species (Abalaka *et al*., 2012). Antibacterial activity of *M. oleifera* leaf extract was due to the presence of a variety of phytochemicals particularly the short polypeptides by inhibiting their cell membrane synthesis and enzyme activity (Bukar *et al*., 2010; Suarez *et al*., 2003). In the present study significant difference in zone of clearance with palmitic acid isolated from *M. oleifera* was observed among different concentration and different bacterial species with P <0.0001. With increasing concentration of palmitic acid the zone of clearance increased. Among the six gram negative and three gram positive bacteria tested against palmitic acid isolated from *M. oleifera* showed that both gram positive and gram negative bacteria are sensitive. Gram negative *K. pneumonia* showed highest zone of inhibition (20.50mm) followed by gram positive *S. pneumonia* and *Bacillus subtilis*. However, Krishnan *et al*., (2016) showed that gram negative bacteria is comparatively more resistant to the plant extract than gram positive bacteria this was also observed in the present study where *P. aeruginosa* as more resistant followed by *P. mirabilis, E. coli* and *S. typhi. P. aeruginosa* was also reported to be highly resistant in the earlier study with hexane (Shafaghat *et al*., 2014). The high resistant by the gram negarive bacteria is due to

lipopolysaccharide (LPS) in the outer cell membrane which has high hydrophobic nature with strong permeability barrier for hydrophobic molecules (Smith-Palmer *et al*., 1998). Whereas gram positive bacteria cell wall contain only peptidoglycan which easily permits the hydrophobic molecule (Lambert *et al*., 2001).

Antifungal activity of palmitic acid also showed effective zone of clearance which highest zone with *C. albicans* and least *F. graminearum* in the present study similar results was observed by (Krishnan *et al*., 2016). Similar to bacteria fungal species also posses' unique cell wall protecting them from cell wall synthesis based on their osmotic nature (Khan and Nasreen, 2010). Zone of inhibition by the compounds is achieved through lysing the cell wall and altering the osmotic pressure of fungal cells.

V. Conclusion

The present research was carried out with an aim to identify different biologically active compounds present in the *M. oleifera* leaf. In this process air dried fresh leaves of *M. oleifera* were dissolved in eight different solvents (Ethanol, Methanol, Acetone, Ethyl Acetate, Petroleum Ether, Chloroform and Benzene) the tested the presence of different secondary metabolites through different methods for each metabolite. In this presence of alkaloids, saponins, proteins, flavonoids, Carbohydrates, Reducing sugar, Tannins, Terpenoids, Phenols, Glycosides and Phytosteroids were tested and found that most of the compounds were with methanol as solvent. Quantification of alkaloids, flavonoids, lignins, tannins, phenols, terpenoids and saponins showed that highest was tannins followed by flavonoids, terpenoids, alkaloids and phenols. The quantities indicted the richness of the *M. oleifera's* medicinal potential. Palmitic acid was isolated through soxhlet apparatus, column chromatography and confirmed with GCMS analysis. Antimicrobial activity of palmitic acid was studied at different concentration for both bacteria and fungi. The results revealed that though both grams negative and positive bacterial species were inhibited the gram negative were resistant particularly *P. aeruginosa* and high zone of inhibition was observed with K. *pneumonia*.

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Table 1. Phytochemical analysis of *M. oleifera* leaf with different solvents

S. No.	Phytochemical test		Solvents						
			Ethanol	Methanol	Acetone	Ethyl Acetate	Petroleum Ether	Chloroform	Benzene
1	Alkaloids	Mayers	+	+	+	+	+	-	-
		Wagners	+	+	+	+	+	-	-
		Dragendroff	+	+	+	+	-	-	-
2	Saponins	Lead acetate	+	+	+	+	-	-	-
		Foam formation	+	+	+	+	-	-	+
3	Protein	Biuret	-	-	-	+	-	-	-
		Millons	+	+	+	+	+	+	+
4	Flavonoids	Alkaline	+	+	+	+	-	-	-
5	Carbohydrates	Molichs	+	+	+	+	+	+	+
	Reducing sugar	Fehling's	-	+	+	+	+	-	+
6	Tannins	Ferric chloride	+	+	+	+	+	+	+
7	Terpenoids	Libermann burchart	+	+	-	+	-	+	-
8	Phenols	Ferric chloride	+	+	+	+	+	-	+
9	Glycosides	Legals	+	+	+	+	+	+	+
10	Phytosteroids	Saulkawsi	+	+	+	+	-	+	-

Table 2. Quantity of secondary metabolites estimated in methanol leaf extract of *Moringa oleifera*

S. No.	Leaf	Quantity g/100gm
1.	Alkaloids	3.29±0.14
2.	Flavanoids	4.67±0.17
3.	Lignins	1.54±0.07
4.	Tannins	6.67±0.11
5.	Phenols	1.64±0.08
6.	Terpenoids	4.27±0.08
7.	Saponins	1.66±0.07

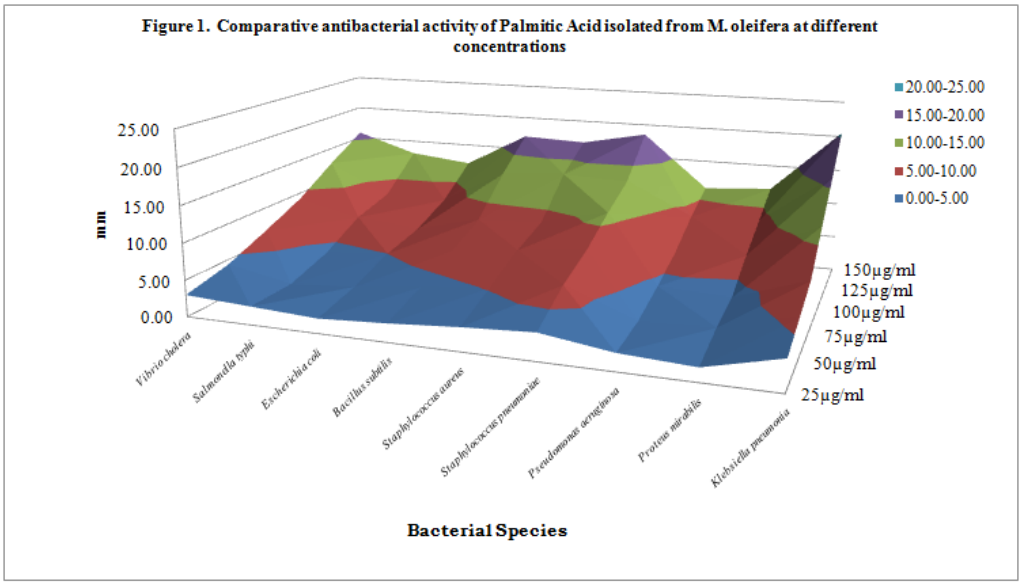

Table 3. Antibacterial activity of Palmitic Acid isolated from *M. oleifera*

S No	Secondary Metabolites	PALMITIC ACID (µg/ml)						*p* value	*f* value
		2.5	**5.0**	**7.5**	**10.0**	**12.5**	**15.0**		
1	***Vibrio cholera***	3.00±0.50	4.33±0.29	6.67±0.29	9.17±0.29	12.67±0.29	15.83±0.29	**< 0.0001**	**657.61**
2	***Salmonella typhi***	2.50±0.50	2.50±0.50	4.17±0.29	7.00±0.50	8.33±0.29	13.00±0.50	**< 0.0001**	**255.48**
3	***Escherichia coli***	2.00±0.50	3.17±0.29	5.00±0.50	6.00±0.50	8.17±0.29	12.00±0.50	**< 0.0001**	**202.46**
4	***Bacillus subtilis***	2.50±0.50	4.17±0.29	6.33±0.29	8.33±0.29	13.00±0.50	17.00±0.50	**< 0.0001**	**545.83**
5	***Staphylococcus aureus***	3.17±0.29	5.17±0.29	7.33±0.29	8.83±0.29	13.67±0.29	16.67±0.29	**< 0.0001**	**941.71**
6	***Staphylococcal pneumoniae***	3.67±0.29	6.50±0.00	8.33±0.29	11.00±0.50	13.33±0.29	18.50±0.50		
7	***Pseudomonas aeruginosa***	2.33±0.29	2.83±0.29	4.33±0.29	6.17±0.29	8.83±0.29	10.83±0.29	**< 0.0001**	**410.91**
8	***Proteus mirabilis***	1.83±0.29	3.17±0.29	3.83±0.29	6.67±0.29	9.00±0.50	11.50±0.50	**< 0.0001**	**300.45**
9	***Klebsiella pneumonia***	4.17±0.29	5.67±0.29	7.17±0.29	11.67±0.29	16.00±0.50	20.50±0.50	**< 0.0001**	**883.70**
p value		**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**		
f value		**11.386**	**61.524**	**76.412**	**91.079**	**178.66**	**178.24**		

Table 4. Antifungal activity of Palmitic Acid isolated from *M. oleifera*

S. No.	Palmitic Acid (25µg/ml)	*Candida albicans*	*Aspergillus fumigates*	*Fusarium graminearum*	*p* value	*f* value
1.	**2.5**	3.67±0.58	2.67±0.29	2.33±0.29	**0.0171**	**8.654**
2.	**5.0**	5.50±0.50	4.17±0.29	3.17±0.29	**0.0008**	**29.404**
3.	**7.5**	8.17±0.29	5.33±0.29	4.17±0.29	**< 0.0001**	**151.08**
4.	**10.0**	11.00±0.50	7.33±0.29	5.83±0.29	**< 0.0001**	**152.25**
5.	**12.5**	14.50±0.50	9.17±0.29	8.17±0.29	**< 0.0001**	**249.20**
6.	**15.0**	19.50±0.50	11.67±0.29	10.5±0.5	**< 0.0001**	**368.97**
***p* value**		**< 0.0001**	**< 0.0001**	**< 0.0001**		
***f* value**		**441.62**	**397.48**	**264.46**		

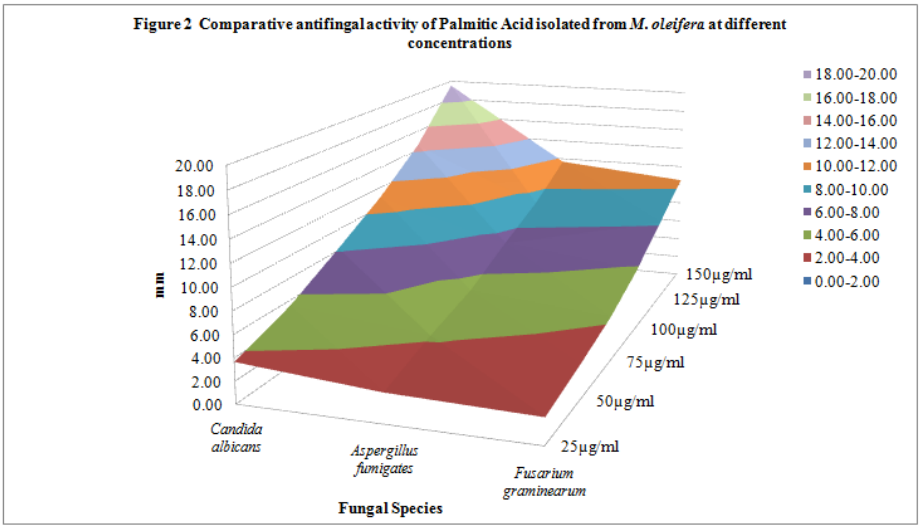

Figure 2 Comparative antifingal activity of Palmitic Acid isolated from *M. oleifera* at different concentrations

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