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Original article

Anticandidal and anti-carcinogenic activities of *Mentha longifolia* (Wild Mint) extracts in vitro

Mohamed Taha Yassin, Ashraf A. Mostafa *, Abdulaziz A. Al-Askar

Botany and Microbiology Department, College of Science, King Saud University, P.O. 2455, Riyadh 11451, Saudi Arabia

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ABSTRACT

Vulvovaginal candidiasis is a common health problem affecting about 75% of women at reproductive age. Teratogenicity and fetal toxicity resulting from antifungal therapy during pregnancy necessitates formulation of novel therapeutic agents. Disk diffusion method was performed to evaluate the antifungal activity of *Mentha longifolia* extracts against different candidal strains. Moreover, the anticancer activity of different *M. longifolia* extracts against MCF7 breast cancer cell line was evaluated by MTT assay. The *M. longifolia* n-hexane extract exhibited the highest antifungal activity, while the methanolic extract showed no antimicrobial activity against the tested *Candida* strains. Minimum inhibitory concentration (MIC) of n-hexane extract was 250 μg/disk against *C. tropicalis* while it was 500 μg/disk against *C. albicans* and *C. glabrata*. Minimum fungicidal concentration (MFC) was 0.5 mg/disk against *C. albicans* and *C. tropicalis*, and 1 mg/disk against *C. glabrata*. GC–MS analysis of the n-hexane extract was performed to detect the active phytochemical components. Menthone (18.37%) was the main phytochemical active component of n-hexane extract followed by butyloctanol (16.13%), 1,5-(1-Bromo-1-methylethyl)-2-methyl-2-cyclohexen-1-one (14.89%), α-tocopherol (13.13%), eugenol (12.21%), α-resorcylamide (11.56%), citronellal (3.71%), butylated hydroxytoluene (3.21%), isocaryophyllene (2.81%), 2-hexyldecanol (2.12%) and tau-cadinol (1.87%). The methanolic extract exhibited the highest anticancer activity against MCF7 breast cancer cell line while diethyl ether extract exhibited the lowest activity with IC_{50} of 91.67 and 244.70 μg/ml respectively. *M. longifolia* extracts could be used as a potential source of novel anticandidal and anti-carcinogenic therapeutic agents.

1. Introduction

Vulvovaginal candidiasis (VVC) is a frequent vaginal infection that commonly occurs among women of childbearing age (Adesiji et al., 2011; Gandhi et al., 2015). About 6–9% of women have suffered from recurrent vulvovaginal candidiasis during their lifetime (Foxman et al., 2013). *Candida albicans* is the most frequent fungal pathogen, causing 85–95% of vaginal candida infections (Hong et al., 2014; Behzadi et al., 2015). Symptoms of VVC include: soreness, itching, burning, and abnormal vaginal discharge (Barousse et al., 2005). Apart from *C. albicans*, *C. tropicalis* and *C. glabrata* were reported as the most frequent strains associated to VVC (Jackson et al., 2005; Boatto et al., 2007; Mohanty et al., 2007; Rad et al., 2011). Virulence factors of *Candida* species that contributes to fungal pathogenicity include hyphal formation, biofilm formation, and extracellular hydrolytic enzyme production (Kumamoto and Vinces 2005; Ramage et al., 2009; Silva et al., 2012). Pregnant women are more susceptible to vaginal candida infections than non-pregnant women (Grigoriou et al., 2006; Ahmad and Khan 2009; Vijaya et al., 2014). Several researchers attributed the high infection rate of VVC among pregnant women to the high hormonal secretion that may promote yeast growth (Babić and Hukić 2010). However, treating pregnant women with antifungals is still challenging arised from the fetal toxicity and altered maternal pharmacokinetic parameters that may affect drug efficacy or increase toxicity to both the mothers and fetuses (Pilmis et al., 2014). There have been increasing reports stating that candida

* Corresponding author at: Botany and Microbiology Department, College of Science, P.O. Box 2455, Riyadh 11451, Saudi Arabia.
E-mail addresses: ashraf812@yahoo.com (A.A. Mostafa), aalaskara@ksu.edu.sa (A.A. Al-Askar).

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species, especially *C. glabrata,* have become resistant to different conventional antifungal drugs (Arendrup and Patterson 2017). Azole resistant strains were reported recently as a main cause of candidal infections (Whaley et al., 2017). Medicinal plants represent a potential source of novel therapeutic agents, being also the basis of indigenous healing systems, still widely used by the majority of populations in many countries (Malterud 2017). *Mentha longifolia* (L.) Huds. (family Lamiaceae) is a medicinal plant, commonly known as wild mint, that possess various pharmacological activities (Naghibi et al., 2010; Mokaberinejad et al., 2012). Previous studies have reported that *M. longifolia* extracts have a potential antimicrobial activity (Asekun et al., 2007; Gulluce et al., 2007). The essential oil of *M. longifolia* showed antifungal activity against *C. albicans* at a concentration of 7120 μg/ml (Mimica-Dukić et al., 2003). Such result was confirmed by Džamić et al., 2010 who reported that antifungal activity of the wild mint essential oil had minimum inhibitory (MIC) and minimum fungicidal (MFC) concentration values of 2.5 and 5 μl/ml, respectively. Natural products have been reported as a promising source for the production of novel anti-cancer therapeutic agents (Gordaliza 2007; Newman 2008).

Regarding to the high incidence of resistant candida strains and the teratogenic hazards of antifungal treatment during pregnancy, novel therapeutic agents for VVC are needed. The present study aimed to evaluate the antifungal efficiency of different *M. longifolia* extracts against the etiological agents of candidal vaginitis, investigate the anti-cancer activity of *M. longifolia* extracts against MCF-7 human breast cancer cell line and detect the phytoactive components of the different *M. longifolia* extracts.

2. Materials and methods

2.1. Preparation of plant extracts

Leaves of *M. longifolia* were obtained from a local market in Riyadh, Saudi Arabia. Identification of plant material was performed by the Saudi herbarium of the Botany Department, College of Science, King Saud University, where vouchers were deposited (KSU_14683). Extraction procedure was performed using four organic solvents (diethyl ether, ethyl acetate, methanol and n-hexane) with different polarities to allow extraction of all phytochemical active components. Leaves of *M. longifolia* were disinfected using 0.5% sodium hypochlorite (NaOCl), washed using sterile distilled water for three consecutive times, and allowed to dry. Leaves were then macerated using a mechanical mortar to obtain a homogenous powder. 50 g of plant powder were soaked into four 500 mL Erlenmeyer flasks, each containing 200 mL of the four different organic solvents. Each flask was stirred over a magnetic stirrer at 25 °C for 48 h and then centrifuged at 9000 rpm for 10 min to get rid of plant residues. Supernatant of each solvent was filtered using Whatman filter paper no. 1 to attain clear filtrates. Finally, the clear filtrates were concentrated using a rotatory evaporator and kept at 4 °C until use. Calculation of the extract yields was carried out according to the following equation: Extract yield = (R/S) × 100;Where R is the weight of the plant extract residue and S is the weight of raw plant sample.

2.2. Fungal strains

Candidal strains (*C. albicans*, *C. tropicalis* and *C. glabrata*) were obtained from the culture collection of Botany and Microbiology Department, College of Science, King Saud University. The fresh inoculum was prepared by subculturing each *Candida* species onto Sabouraud dextrose agar (SDA) medium at 35 °C for 48 h.

2.3. Preparation of candidal inocula

Previously prepared SDA slants were inoculated with different *Candida* species. The *Candida* specimens grown on the SDA slants were harvested using a sterile saline solution. The absorbance of microbial suspension was adjusted at 560 nm using a spectrophotometer to obtain a viable cell count of 10^7 colony forming unit (CFU)/mL for each Candida species.

2.4. Screening of the antifungal activity of different M. longifolia extracts

The disk diffusion method was performed to evaluate the antifungal activity of different *M. longifolia* extracts against different *Candida* strains. *M. longifolia* extracts were screened for their anticandidal activity in which 15 mL of SDA medium was poured into sterile petri dishes (base layer) followed by 10 mL of SDA containing a *Candida* suspension (1 mL of *Candida* for each 100 mL of medium to obtain a viable cell count of 10^5 CFU/mL). Sterile filter paper disks (8 mm in diameter) were loaded with 10 mg of *M. longifolia* extracts then placed over the seeded layer once it had solidified (Mansourian et al., 2014; Mostafa et al., 2018). Fluconazole antifungal drug was used as a positive control with a concentration of 25 μg/disk according to the Clinical & Laboratory Standards Institute (CLSI, 2007). Fungal susceptibility to fluconazole, as an antifungal drug, was determined based on the inhibition zone diameter as follows: for *C. albicans* and *C. tropicalis, a diameter* $\geq$19 mm was interpreted as sensitive, 15–18 mm as dose dependent, and $\leq$14 mm as resistant; while for *C. glabrata*, a diameter $\geq$15 mm was interpreted as dose dependent, and finally a diameter $\leq$14 mm was interpreted as resistant. Plates were incubated at 4 °C for 2 h to allow the *M. longifolia* extracts to diffuse throughout the medium. Finally, plates were incubated at 35 °C for 48 h and the inhibition zone diameter was measured using Vernier caliper. Inhibition zone diameters were recorded and considered as an indication of antifungal efficiency of the mint extracts against different candidal pathogens.

2.5. Determination of the minimum inhibitory concentration (MIC)

The lowest concentration of *M. longifolia* extract exhibiting anticandidal activity was identified as the minimum inhibitory concentration (MIC). The MIC was evaluated for the n-hexane extract of *M. longifolia* as it showed the highest antimicrobial efficiency. For such, 15 mL of SDA medium was poured into sterile petri dishes (base layer) and 10 mL of seeded SDA was poured on top and left to solidify. Sterile filter paper disks (8 mm in diameter) with different concentration of n-hexane extracts of *M. longifolia* (62.5, 125, 250, 500, 1000, 2000 μg/disk) were placed over the seeded medium. Plates were stored at 5 °C for 2 h to allow the mint extracts to diffuse. Finally, plates were incubated at 35 °C for 48 h and the inhibition zone diameters were measured. MIC was recorded for the different *Candida* strains tested.

2.6. Determination of the minimum fungicidal concentration (MFC)

The lowest concentration of the *M. longifolia* n-hexane extract in which pathogens show no growth is defined as MFC. Freshly prepared SDA plates were inoculated with streaks taken from the inhibition zone area of the MIC and the two successive concentrations. Plates were then incubated at 35 °C for 48 h and examined for candidal growth. The lowest concentration of the n-hexane extract of *M. longifolia* showing no *Candida* growth was recorded as the minimum fungicidal concentration (MFC).

2.7. Cytotoxicity assay

Human breast cancer (MCF7) cell lines were obtained from the collection of the zoology department, college of science, King Saud University, Saudi Arabia. The anti-cancer activity of *Mentha longifolia* (diethyl ether, ethyl acetate, methanolic and n-hexane) extracts against MCF7 cell lines was evaluated using the 3-(4,5-dimethyl thiazol)-2,5-diphenyl tetrazolium bromide (MTT) assay (Famuyide et al., 2019). The cells were cultured in minimal essential medium (MEM, Highveld Biological, South Africa) supplemented with 0.1% gentamicin (Virbac) and 5% foetal calf serum (Adcock-Ingram) in a 5% CO_2 incubator. Incubation of MCF7 cells in 96-well plate at 37 °C for 24 hr. in 5% CO_2 incubator to allow adherence of cells. The crude *M. longifolia* extracts were dissolved in methanol (10 mg/mL), and appropriate dilutions were prepared. MCF7 cells were treated with the *M. longifolia* extracts at concentration range of (0.0065–1 mg/ml). After a 48hr exposure, removal of supernatant was performed and MTT solution was added to the wells at a concentration of 5 mg/ml. Incubation of plates at 37 °C for 4 hr and then supernatants were removed. Finally, 50 µl of DMSO was added to the wells to allow the solubilization of the formed formazan crystals. Absorbance was measured at wavelength of 570 nm. The concentration causing 50% inhibition of cell viability (IC_{50}) was calculated.

2.8. Gas chromatography–mass spectrometry (GC–MS) of M. longifolia extracts

Phytochemical analysis of the extracts was carried out using (an Agilent 7890 gas chromatograph and an Agilent 5975C Mass Spectrometer, USA) to investigate the phytochemical active components of different *M. longifolia* extracts. The gas chromatograph was set as follows: a HP-5MS UI capillary column (30 m × 0.25 m m; 0.25 µm film thickness), helium carrier gas with a flow rate of 1 mL/min, an oven temperature of 40 °C and adjusted to 250 °C at 5 °C/min, an injection volume of 1 µl, injector and detector temperatures of 250 °C, and a split ratio of 1:50. The mass spectrometry was set as follows: an ionization potential of 70 eV, a mass range from 40 to 400 amu, and an electron multiplier energy of 2000 V. Identification of the phytochemical components of leaf extracts of *M. longifolia* was determined by comparing the results of the GC–MS analysis with the reference retention time and spectral mass data of the NIST database.

3. Results and discussion

3.1. Extract yields

The highest yield of *M. longifolia* extracts was obtained using methanol (5.49%) as solvent, followed by diethyl ether (3.82%), ethyl acetate (1.27%), and n-hexane (0.29%).

3.2. Antifungal activity

In our current study, *C. albicans* strain expressed resistance to fluconazole and that was coincident with the results reported by Nasrollahi et al., (2015) in which 94% of *C. albicans* strains isolated from vaginitis patients were resistant. Moreover, the resistance of *C. glabrata* strain to fluconazole drug was detected and the previous result was similar to that of Hasanvand et al., (2017) who reported the fluconazole resistance of three *C. glabrata* strains isolated from vaginitis patients.

Screening of the antifungal potency of ethyl acetate, diethyl ether and n-hexane extracts of *M. longifolia* exhibited a high efficiency at concentration of 10 mg/disc against different candidal strains with different susceptibility patterns. The *M. longifolia* extract using n-hexane as a solvent showed the highest antifungal activity against *C. albicans*, *C. glabrata*, and *C. tropicalis*, with inhibition zone diameters of 27.43, 16.33, and 22.63 mm, respectively (Table 1). These results were in accordance with that of Carretto et al. (2010) who proved the antimicrobial activity of *Mentha* sp. against *Candida* strains at concentration range of 7.8–250 mg/ml with inhibition zone diameters ranging from 5 to 10 mm.

On the other hand, the methanolic extracts of *M. longifolia* exhibited no antifungal activity against the three *Candida* strains which was ascertained by Gulluce et al., (2007) who found that methanolic extracts of *M. longifolia* leaves have no antimicrobial activity against *C. albicans*. In contrast, the diethyl ether extract of *M. longifolia* showed high antifungal activity against all three *Candida* strains with inhibition zone diameters of 14.93, 12.17, and 21.67 mm for *C. albicans*, *C. glabrata*, and *C. tropicalis*, while a moderate efficacy was detected for the ethyl acetate extract against the tested strains with inhibition zone diameters of 13.43, 13.20, and 16.57 mm respectively. Furthermore, the antifungal activity of the n-hexane extract of *M. longifolia* against non-*albicans* strains (*C. glabrata* and *C. tropicalis*) was significantly higher ($p < 0.05$) than that of fluconazole (control).

3.3. Determination of the minimum inhibitory concentration (MIC) of the M. longifolia n-hexane extract

The n-hexane extract of *M. longifolia* was the most effective extract against the concerned *Candida* strains and its MIC against *C. albicans* was 500 µg/disk, a value lower than that previously ascertained by Bakht et al., (2014) who reported MIC value by 2 mg/disk. MIC of n-hexane extract against non-*albicans* strains (*C. tropicalis* and *C. glabrata*) was 250 and 500 µg/disk respectively (Table 2). MIC results confirmed that *C. tropicalis* exhibited the highest sensitivity to the n-hexane extract of *M. longifolia* (Fig. 1).

3.4. Determination of the minimum fungicidal concentration (MFC) of the M. longifolia n-hexane extract

The n-hexane extract of *M. longifolia* was tested for MFC against the three *Candida* strains used in order to detect the lowest concentration of the extract that shows no fungal growth. The minimum fungicidal concentration was achieved at 0.5 mg/disk against *C. albicans* and *C. tropicalis* while it was 1 mg/disk against *C. glabrata*. These results were contrasted with the previous study which reported the fungicidal activity of *Mentha* sp. extract at concentration range of 0.5–8 µl/mL against *Candida* pathogens (Saharkhiz et al., 2012).

3.5. Cytotoxicity assay

Methanolic extract exhibited the highest cytotoxic activity against MCF7 breast cancer cell line with IC_{50} of 91.67 µg/ml (Fig. 2). Our results were in accordance with that of Al-Ali et al.,

Table 1
Antimicrobial activity of the *M. longifolia* extracts (10 mg/disc) against three *Candida* species.

M. longifolia extracts (10 mg/disc)	Inhibition zone diameter (mm) of *Candida* pathogens		
	C. albicans	*C. glabrata*	*C. tropicalis*
Diethyl-ether extract	14.93 ± 0.15	12.17 ± 0.14	21.67 ± 0.43
Ethyl acetate extract	13.43 ± 0.32	13.20 ± 0.23	16.57 ± 0.49
Methanolic extract	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
N-hexane extract	22.63 ± 0.67	16.33 ± 0.29	27.43 ± 0.32
Fluconazole (25 µg/disc)	13.77 ± 0.31	9.67 ± 0.42	23.87 ± 0.37

Table 2
Minimum inhibitory concentration (MIC) of the *M. longifolia* n-hexane extract against three *Candida* species.

Concentration of plant extract (μg/disc)	Inhibition zone diameter (mm) of *Candida* strains		
	C. albicans	*C. glabrata*	*C. tropicalis*
62.5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
125	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
250	0.00 ± 0.00	0.00 ± 0.00	11.40 ± 0.17
500	13.17 ± 0.28	9.63 ± 0.07	15.38 ± 0.23
1000	17.27 ± 0.24	12.35 ± 0.32	21.65 ± 0.26
2000	20.97 ± 0.12	14.25 ± 0.20	23.97 ± 0.27

(2013) who reported that the methanolic extract of *M. longifolia* possess potential anti-cancer activity against MCF7 cell line at concentration range of 20–320 μg/ml. Diethyl ether extract exhibited the lowest activity against MCF7 cell line with IC_{50} of 244.7 μg/ml. Moderate cytotoxic activities of ethyl acetate and n-hexane extracts were detected with IC_{50} of 136.3 and 183.2 μg/ml respectively. Our results were in accordance with that of Sharma et al. (2014) who reported the anticancer activity of *Mentha* sp. extracts against eight human (breast, colon, glioblastoma, lung, leukemia and prostate) cancer cell lines. Stringaro et al. (2018) ascertained that *Mentha* spp. extracts were a potential source of novel anticancer agents due to their high activity against different cancer cell lines.

3.6. GC–MS analysis of M. longifolia extracts

Phytochemical analysis of the *M. longifolia* n-hexane extract exhibiting the highest anticandidal activity showed that menthone (18.37%) was the main phytochemical active component followed by butyloctanol (16.13%), 1,5-(1-bromo-1-methylethyl)-2-methyl-2-cyclohexen-1-one (14.89%), α-tocopherol (13.13%), eugenol (12.21%), α-resorcylamide (11.56%), citronellal (3.71%), butylated hydroxytoulene (3.21%), isocaryophyllene (2.81%), 2-hexyldecanol (2.12%), and tau-cadinol (1.87%) (Table 3). These components were similar to that reported by Okut et al. (2017) who reported that the chemical composition of *M. longifolia* extract was compromised of Menthone as a predominant chemical constituent (19.31%) followed by Pulegone (12.42%), Piperitone (11.05%), Dihydrocarvon (8.32%), Limonene (6.1%), 3-Terpinolenone (5.66%), 1,8-Cineole (4.37%), Germacrene D (3.38%) and Caryopyllene (3.19%), respectively. Other study reported that wild mint oil was mainly composed of menthone (34.13%) (Salman et al., 2015). Variation in the chemical composition of wild mint between our findings and previous studies may be attributed to a variety of factors, such as geographic variation, harvest time, environmental and agronomic conditions, the botanical parts of plants, and extraction methods (Kurihara et al., 2003; Fang et al., 2011; Xi et al., 2014; Bessada et al.,2016; Liu et al., 2016).

The reported potential antimicrobial activity of *M. longifolia* can be attributed to the presence of oxygenated monoterpenes in their phytochemical compounds (Mimica-Dukić et al., 2003; Şahin et al., 2003). However, Samber et al. (2015) reported that the main phytochemical components of *Mentha* sp. essential oil are menthone and carvone, and both of them act as antifungal agents due to their effect on ergosterol of cell membrane biosynthesis leading to microbial cell death.

Moreover, the diethyl ether extract of *M. longifolia* that exhibited a moderate anticandidal activity against the tested strains was composed of 3,5-dihydroxybenzamide (42.89%) as a main phytoactive component, followed by 5-(7a-isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-en-1-ol (11.03%), isoledene (10.91%), butylated hydroxytoluene (9.20%), epizonarene (4.92%), lycopersen (5.27%), epizonarene (4.92%), pyrrolo [3,2-d]pyrimidin-2,4(1H,3H)-dione (3.69%), naphthalene,1,1/-(1,10-decanediyl)bis*decahydro (3.37%), 1,1-naphthyl isocyanide (2.84%), benzenemethanol,3-hydroxy-5-methoxy- (2.09%), phosphinic acid, diisopropyl-,menthyl ester (1.89%), and phytol acetate (1.89%) (Table 4).

The *M. longifolia* ethyl acetate extract that exhibited a moderate antifungal activity against the concerned *Candida* strains whose

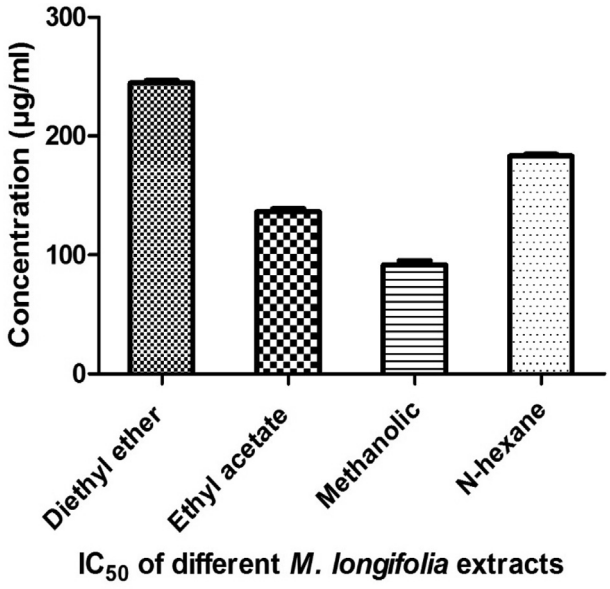

Fig. 2. IC_{50} of different *M. longifolia* extracts against MCF7 cancer cell line.

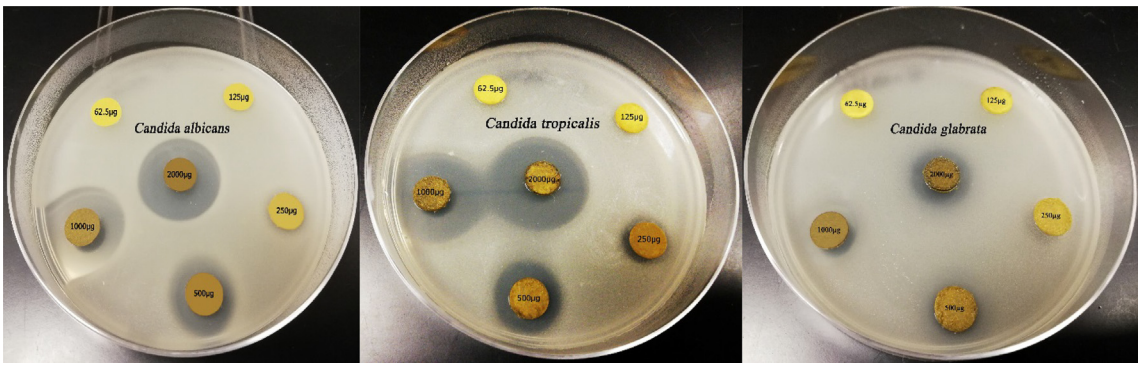

Fig. 1. Minimum inhibitory concentration (MIC) of the *Mentha longifolia* n-hexane extract against the three *Candida* pathogens tested.

Table 3
Phytochemical components of the *M. longifolia* n-hexane extract.

Compounds	Chemical formula	M.W	RT	% of Total
Menthone	$C_{10}H_{18}O$	154.16	4.699	18.37
Citronellal	$C_{10}H_{18}O$	182.30	4.911	3.71
α-Resorcylamide	$C_7H_7NO_3$	154.25	6.433	11.56
1,5-(1-Bromo-1-methylethyl)-2-methyl-2-cyclohexen-1-one	$C_{10}H_{15}BrO$	153.14	8.604	14.89
Eugenol	$C_{10}H_{12}O_2$	245.16	8.923	12.21
Isocaryophyllene	$C_{15}H_{24}$	164.20	10.508	2.81
Butylated Hydroxytoulene	$C_{15}H_{24}O$	204.36	12.681	3.21
T-cadinol	$C_{15}H_{26}O$	222.37	16.130	1.87
Butyloctanol	$C_{12}H_{26}O$	290.50	26.829	16.13
α-Tocopherol	$C_{29}H_{50}O_2$	224.34	32.707	13.12
2-Hexyldecanol	$C_{16}H_{34}O$	376.6	35.794	2.12

GC–MS analysis showed that piperitone (15.75%), menthone (15.28%), and citronellal (14.87%) were the main active components (Table 5). Other study attributed the potent anticandidal activity of *M. longifolia* to their constituents of citronellal which could be used as a chemotherapeutic agent for the treatment of VVC or RVVC (Medeiros et al., 2017). The n-hexane and ethyl acetate extracts of *M. longifolia* were rich in menthone with percentage of 18.37% and 15.28% respectively. Menthone is a phenolic monoterpene that was reported to possess hydrophobic characteristics causing disruption of microbial selective permeability

Table 4
Phytochemical components of the *M. longifolia* diethyl ether extract.

Compounds	Chemical formula	M.W	RT	% of Total
Benzenemethanol,3-hydroxy-5-methoxy-	$C_8H_{10}O_3$	154.16	4.891	2.09
Phosphinic acid, diisopropyl-,menthyl ester	$C_{16}H_{33}O_2P$	288.41	5.141	1.89
Naphthalene,1,1/-(1,10-decanediyl)bis*decahydro	$C_{30}H_{54}$	414.70	5.211	3.37
3,5-Dihydroxybenzamide	$C_7H_7NO_3$	153.14	6.978	42.89
1,1-Naphthyl isocyanide	$C_{11}H_7N$	153.18	7.410	2.84
Pyrrolo[3,2-d]pyrimidin-2,4(1H,3H)-dione	$C_6H_5N_3O_2$	151.12	9.240	3.69
Lycopersen	$C_{40}H_{66}$	547.00	10.940	5.27
Butylated Hydroxytoluene	$C_{15}H_{24}O$	220.35	12.959	9.20
Isoledene	$C_{15}H_{24}$	204.36	13.067	10.91
Epizonarene	$C_{15}H_{24}$	204.36	16.544	4.92
5-(7a-isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-en-1-ol	$C_{20}H_{34}O$	290.50	27.120	11.03
Phytol acetate	$C_{22}H_{42}O_2$	338.57	34.075	1.89

Table 5
Phytochemical components of the *M. longifolia* ethyl acetate extract.

Compounds	Chemical formula	M.W	RT	% of Total
3-Hepten-2-one, 3-ethyl-4-methyl-	$C_{10}H_{18}O$	154.25	4.891	4.04
Menthone	$C_{10}H_{18}O$	154.25	4.991	15.28
Citronellal	$C_{10}H_{18}O$	154.25	5.103	14.87
Endo-3-acetamido camphor	$C_{10}H_{18}O$	154.25	6.806	7.02
Piperitone	$C_{10}H_{16}O$	152.23	6.847	15.75
Pyrrolo[3,2-d]pyrimidin-2,4(1H,3H)-dione	$C_6H_5N_3O_2$	151.12	8.822	14.80
Menthol crotonate	$C_{14}H_{24}O_2$	224.33	10.636	1.71
Isoledene	$C_{15}H_{24}$	204.36	12.018	4.97
γ-Muurolene	$C_{15}H_{24}$	204.36	12.820	3.78
T-Cadinol	$C_{15}H_{26}O$	222.36	16.223	9.95
5-[7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl]-3-methyl-pent-2-en-1-ol	$C_{21}H_{44}O_3S$	376.60	26.887	7.82

Table 6
Phytochemical components of *M. longifolia* methanolic extract.

Compounds	Chemical formula	M.W	RT	% of Total
Menthone	$C_{10}H_{18}O$	154.25	4.777	9.36
Decalin,1-methoxymethyl-	$C_{12}H_{22}O$	182.30	4.995	5.29
Isothujol	$C_{10}H_{18}O$	154.25	5.358	6.62
3,5-Dihydroxybenzamide	$C_7H_7NO_3$	152.23	6.648	22.33
2-Adamantanol,2-(bromomethyl)-	$C_{11}H_{17}BrO$	245.16	8.819	7.98
Eugenol	$C_{10}H_{12}O_2$	164.20	9.022	6.09
Caryophyllene	$C_{15}H_{24}$	204.36	10.541	11.30
T-Cadinol	$C_{15}H_{26}O$	222.36	16.206	10.72
5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-en-1-ol	$C_{20}H_{34}O$	290.50	26.862	6.56
Menthol crotonate	$C_{14}H_{24}O_2$	224.34	29.768	5.51
Sulfurous acid, hexyl pentadecyl ester	$C_{21}H_{44}O_3S$	376.60	30.959	8.22

through its incorporation into microbial cell membranes (Saharkhiz et al., 2012).

The methanolic extract of *M. longifolia* exhibited the highest cytotoxic activity against MCF7 breast cancer cell line so can be used as a potential source of novel anticancer therapeutic agents. The main phytoactive compounds of methanolic extract were caryophyllene, t-cadinol, menthone, eugenol and menthol crotonate (Table 6). The potential anti-cancer activity of *M. longifolia* extracts may be attributed to their constituents of eugenol which possess the ability to induce apoptosis against the cancer cells Jaganathan and Supriyanto (2012).

4. Conclusions

Mentha longifolia extracts can be considered as a potential source of natural antifungal drugs where the tested extracts showed a highly antifungal potency- against fluconazole resistant strains like *C. albicans* and *C. glabrata*. Hexanic extract of *M. longifolia* exhibited the highest antifungal activity against all tested candidal strains compared with the other solvents. Moreover, these solvent extracts of *M. longifolia* were found to possess a potent anticancer activity against MCF7 human breast cancer cell line. The potential anti-*Candida* and anti-Cancer activities of *M. longifolia* extracts may be attributed to their chemical constituents of phytoactive components such as menthone, citronellal and eugenol. From the all solvent extracts, methanolic extract showed the highest cytotoxic activity against human breast cancer cells. Results of the anticandidal and anticancer activities highlight the potential of utilizing these extracts as a safe and effective antifungal and anti-cancer therapeutic agents.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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