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## Chemical constituents of *Mentha piperita* and *Pongamia pinnata* essential oils and their synergistic anticandidal activity with some antibiotics against multidrug resistant clinical isolates of *Candida*

Tejas Rathod, Hemali Padalia and Sumitra Chanda

### Abstract

**Objective:** The aim of the present work was to evaluate anticandidal activity of *Mentha piperita* and *Pongamia pinnata* essential oils and synergistic anticandidal activity of these essential oils with some commonly used commercial antibiotics like Amphotericin B, Nystatin, Fluconazole, Ketoconazole, Clotrimazole and Itraconazole against multidrug resistant clinical isolates of *Candida*.

**Method:** The synergistic effect of antifungal activity was evaluated against clinical isolates by disc diffusion assay.

**Results:** The synergistic activity of the antibiotics with *Piper mentha* essential oil was better than with that of *Pongamia pinnata* essential oil. *Piper mentha* essential oil increased the anti-candidal effect of azole antibiotics Fluconazole and Ketoconazole, while it had less synergistic effect on Clotrimazole and Itraconazole.

**Conclusion:** The essential oil of *Piper mentha* essential oil in combination with antibiotics could be used as an interesting and alternative source of anticandidal agent against *Candida* species.

**Keywords:** *Piper mentha*; *Pongamia pinnata*; Essential Oils; Synergistic; Ketoconazole; *Candida*, clinical isolates

### 1. Introduction

Fungal diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide [1] and a steady increase in the occurrence of fungal infections has been observed globally. Fungal infections have been gaining prime importance because of the morbidity of hospitalized patients. *Candida* is the most important causative agent of opportunistic fungal infections and a rising problem worldwide. The genus *Candida* includes hundreds of species of which over 40 have been recovered from human samples [2] and implicated in life-threatening infections, particularly in immune compromised hosts. This (*C. albicans*) most wide-spread opportunistic pathogenic fungus, has a high degree of flexibility and can exist and proliferate in environments that are extremely variable in oxygen and carbon dioxide levels, pH, osmolarity, availability of nutrients, and temperature [3,4]. Nowadays, the increased prevalence of antibiotic therapy, human immunodeficiency virus (HIV) infection and immunosuppressive disease have led humans to become more susceptible to *Candida* infection, with chief fungal pathogen *Candida albicans* [5].

The antifungal antibiotics that are generally used to treat candidiasis or *Candida* infections are polyene antibiotics (Nystatin, Amphotericin B), the azole derivatives (Fluconazole, Ketoconazole, Clotrimazole and Itraconazole), the allylamines (Terbinafine) and thiocarbamates, the morpholines and the nucleoside analogs [6]. But use and overuse or misuse has led for the development of antibiotic resistant strains. The antibiotics which were working once upon a time are no more as sensitive as before. The rising problem of multi drug resistant strains and increasing occurrence of infectious diseases has necessitated the search for alternative cure. The best answer found was the use of natural products. There has been a revival of interest in the use of medicinal and aromatic plants for the treatment of infectious diseases especially because they have been reported to be safe and without any side-effects [7]. The plant extracts alone or in combination and essential oils alone or in combination showed many promising results and are reported for antimicrobial activities [8-12]. Antimicrobial activity of many essential oils is reported by [13, 14].

Considering the above, it was thought of interest to evaluate the synergistic antifungal efficacy of two essential oils viz. *Piper mentha* essential oil and *Pongamia pinnata* essential oil. Both the essential oils were evaluated against a panel of clinical isolates of *Candida* alone and in

combination with six commonly used commercial antibiotics viz. Amphotericin B, Nystatin, Ketoconazole, Fluconazole, Clotrimazole and Itraconazole. The details of the selected essential oils and their reported biological activities are given below.



a) Photograph of *Piper mentha*



b) Photograph of *Pongamia pinnata*

*Mentha piperita* or *Piper mentha* L. (a) belongs to the family Lamiaceae. There are many reported activities of *Piper mentha*. Kalemba and Kunicka, [15] reported antibacterial and antifungal activity; anti-proliferative activity is reported by Manosroi *et al.*, [16]; anti-inflammatory activity is reported by Miguel [17]; antipyretic, antiulcer, anti-diabetic, anticancer activity is reported by Umashanker and Shruti [18]; insecticidal activity is reported by Souto *et al.*, [19]; antioxidant activity is reported by Gharib and da Silva [20]. Singh *et al.*, [21] reported antibacterial and antioxidant activity.

*Pongamia pinnata* Linn. (b) belongs to the family Fabaceae. It is commonly known as Karanja. This plant is an important medicinal plant and various parts show different biological activity [22]. The reported activities of *Pongamia pinnata* are antihyperglycemic and anti lipidperoxidative activity [23], antioxidant, antimicrobial activity [24], antidiabetic activity [25], anticancer activity [26], phytochemical and pharmacognostic study [27], antimicrobial activity [28], antihyperglycemic and antilipidperoxidative effects, antihyperammonemic effect, antifungal and antibacterial activity, antiinflammatory activity, antiviral activity, antifilarial potential, action against infectious diarrhea, nootropic activity, antinociceptive activity, protective effect against nephrotoxicity, ulceroprotective activity [29].

## 2. Materials and methods

### 2.1 Essential oils

The *Piper mentha* essential oil and *Pongamia pinnata*

essential oil was purchased from Yucca Enterprise Mumbai, India.

### 2.2 Antibiotics used

The six different antibiotics (Hexa Disc), Amphotericin B-AMP (100 units/ disc), Nystatin -NYS (100 units/ disc), Ketoconazole-KT (30 mcg/ disc), Fluconazole-FLC (10 mcg/ disc), Clotrimazole-CC (10 mcg/ disc) and Itraconazole -IT (30 mcg/disc) were used in the study.

### 2.3 Fungal strains

Fifty clinical strains of *Candida* spp. were collected from various microbiological laboratories of Rajkot, Gujarat, India. Most of the strains were isolated from patients with skin infections and some from urine sample. The isolates were cultured on Sabouraud dextrose agar medium with Chloramphenicol under aerobic conditions within a temperature range of 28° C. The isolates were identified as *Candida* species on the basis of some biochemical tests like Blastospore / Chlamyospore formation, color of colony on HiChrome *Candida* differential agar, carbohydrate assimilation test (sucrose, mannose, lactose, malate) and negative absorption (urea and nitrate). The susceptibility test revealed 19 isolates as multidrug resistant (MDR) and hence these 19 isolates were used for further study. The 19 isolates are named as C1, C2, C3, C5, C6, C12, C13, C14, C15, C18, C21, C22, C23, C26, C30, C41, C42, C43 and C44.

### 2.4 Synergistic anticandidial assay

Synergistic anticandidial activity of the *P. mentha* essential oil and *P. pinnata* essential oil with antibiotics (Amphotericin B, Nystatin, Ketoconazole, Fluconazole, Clotrimazole, Itraconazole) were evaluated by using disc diffusion method [30]. The Petri plates were prepared by pouring 20 ml Sabouraud dextrose agar seeded with 200 µl test culture containing  $1 \times 10^8$  cfu/ml as McFarland 0.5 turbidity standard. Plates were allowed to solidify. Standard antibiotics paper discs (6 mm) were impregnated with 20 µl of *P. mentha* and *P. pinnata* essential oils (20%) dissolved in ethanol separately. The sterile paper discs were impregnated with 20 µl of *P. mentha* and *P. pinnata* essential oil (in 20% Ethanol) separately. All the discs were allowed to saturate for 30 min and were placed on the surface of the agar plates which had previously been inoculated with *Candida* isolates respectively. All plates were incubated for 48 h at 28 °C. Results were recorded by measuring the zone of inhibition appearing around the discs. All the tests were performed in triplicate and the mean values are presented.

### 2.5 Increase in fold area

Increase in fold area (IFA) was calculated as  $(B^2 - A^2) / A^2$

Where A- inhibition zone for Antibiotics and B - Inhibition zone for essential oil + antibiotics.

### 2.6 Gas-chromatography-mass spectrometry (GC-MS) analysis

The chemical composition of the essential oil was analyzed using GC-MS. The essential oil solution was injected into GC-MS (QP-2010 Plus, Shimadzu). The capillary column was BPX-35 (length=30 m, i.d. = 0.25mm, thickness = 0.25µm). The GC-MS oven temperature was increased from 40° C to 310° C at rate of 10° C/min with final hold time 5 min. The injector and detector temperature were maintained at 300° C and 290° C while spectra recorded in the 40 -700 m/z range and ion source temperature was 200° C. Other operating

condition were as follows: Injection mode: split, Flow control: Liner velocity, Column flow: 0.80ml/min and Split ratio: 0.5 Essential oil components were quantified by relative percent peak area of TIC from the MS signal and identified by comparing their mass fragmentation pattern with those stored in the spectrometer database.

### 3. Results

#### 3.1 Chemical composition *Piper mentha* essential oil

Chromatogram of GC-MS analysis of *P. mentha* essential oil

is given in Fig. 1. The identified compounds of essential oil are listed in Table 1 with their respective retention time and percent compositions. The results of GC-MS analysis of *P. mentha* essential oil led to identification of 9 compounds accounting for the total oil. The principal compound identified were Propylene Glycol (13.27%), Benzyl alcohol (7.95%), p- Menthone (28.33%), Menthol (33.35%), Naphthalene (7.43%). Several other compounds such as Cyclohexanol (0.82%), beta- Elemene (0.71%), Cadinene (2.21%) and D-Limonene (0.49%) were in less amounts.

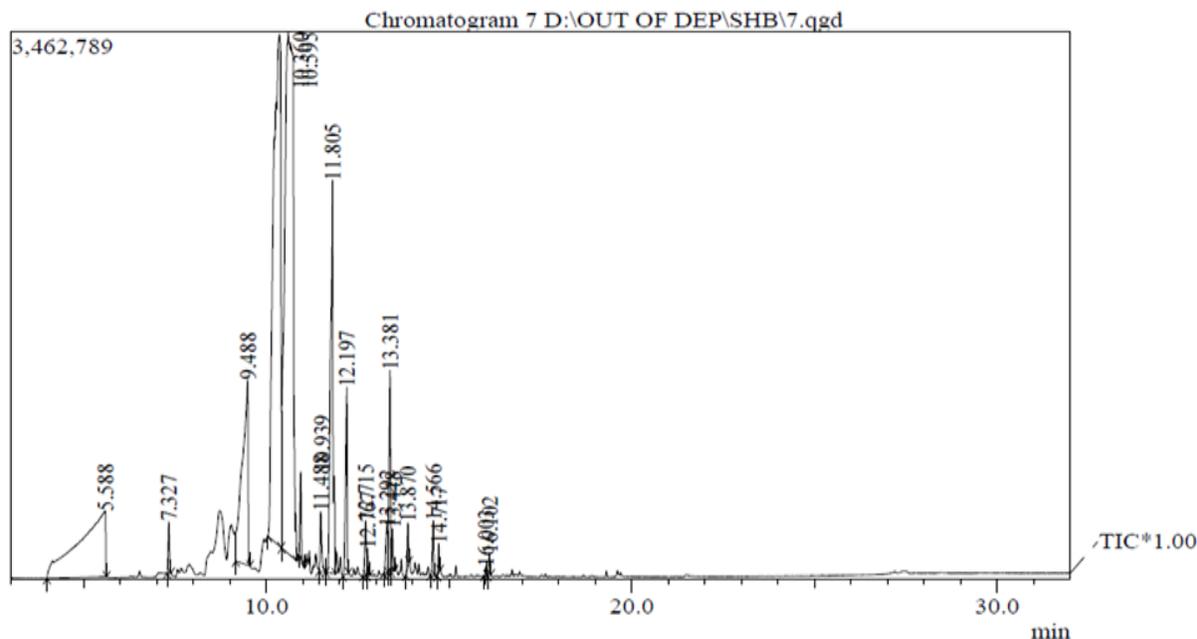
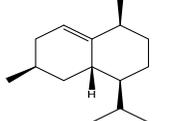


Fig 1: Chromatogram of GC-MS analysis of *Piper mentha* essential oil

Table 1: Chemical composition, retention time, structure and % area of *Piper mentha* essential oil

No.	Retention Time	% Area	Compound Name	Structures
1	5.588	13.27	Propylene Glycol	
2	7.327	0.49	D-Limonene	
3	9.488	7.95	Benzyl alcohol	
4	10.360	28.33	p- Menthone	
5	10.595	33.35	Menthol	
6	10.939	0.82	Cyclohexanol	
7	11.488	0.71	beta- Elemene	
8	11.805	7.43	Naphthalene	

9	12.97	2.21	Cadinene	
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### 3.2 Chemical composition *Pongamia pinnata* essential oil

Chromatogram of GC-MS analysis of *P. pinnata* essential oil is given in Fig. 2. The identified compounds of essential oil are listed in Table- 2, with their respective retention time and percent compositions. The results of GC-MS analysis of *P. pinnata* essential oil led to identification of 16 compounds accounting for the total oil. The principal compound identified were Oleic acid (21.47%), 9-Octadecenoic acid, methyl ester, (E)- (19.79%), 2H-Isoindole-2-acetic acid, 1,3-dihydro-1,3-dioxo-, ethyl ester (10.58%), 2-[5-(2-Methylbenzooxazol-7-yl)-1H-pyrazol-3-yl]-phenol (13.17%),

Pyridazin-3(2H)-one, 4,5-di(ethylthio)-2-phenyl- (6.07%). Several other compounds such as Methyl stearate (1.88%) Hexadecanoic acid, methyl ester (2.70%), Octadecanoic acid (2.49%), 1-Hexyl-2-nitrocyclohexane (0.43%), Heneicosanoic acid, methyl ester (0.42%), 1-Hexyl-2-nitrocyclohexane (0.50%), Ethanone, 1-(4-cyclohexylphenyl)- (1.05%), 3H-Pyrazol-3-one, 4-[[4-(diethylamino) phenyl]imino]-2,4-dihydro-5-methyl-2-phenyl (3.90%), Benzamide, N-methyl-4-(4-methyl-1-phthalazinylamino)- (1.13%), Diazene, bis[2,6-bis(1-methylethyl)phenyl]- (0.64%), n-Hexadecanoic acid (2.64%) were in less amount.

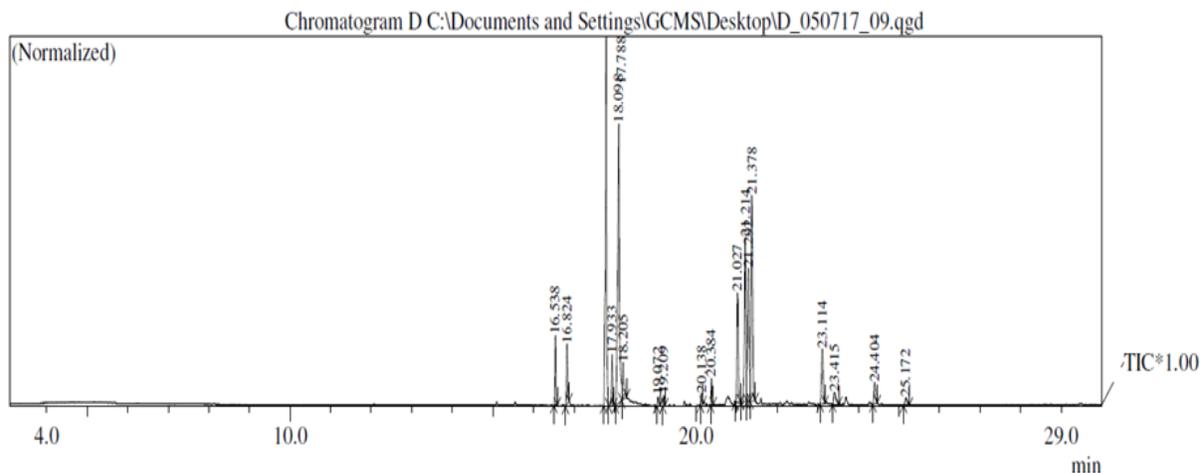
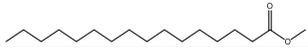
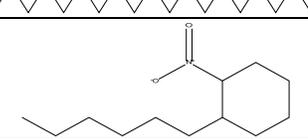
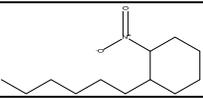
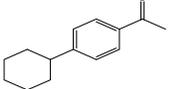
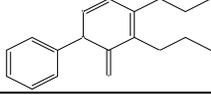
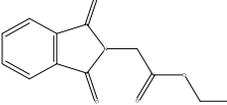
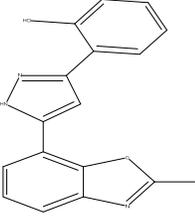
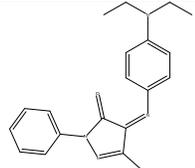
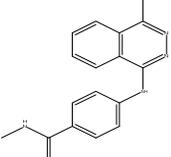
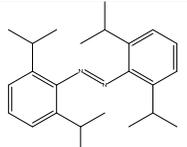


Fig 2: Chromatogram of GC-MS analysis of *Pongamia pinnata* essential oil

Table 2: Chemical composition, retention time, structure and % area of *Pongamia pinnata* k essential oil

No.	Retention time	%Area	Compound Name	Structures
1	16.538	2.70	Hexadecanoic acid, methyl ester	
2	16.824	2.64	n-Hexadecanoic acid	
3	17.788	19.79	9-Octadecenoic acid, methyl ester, (E)-	
4	17.933	1.88	Methyl stearate	
5	18.098	21.47	Oleic Acid	
6	18.205	2.49	Octadecanoic acid	
7	19.072	0.43	1-Hexyl-2-nitrocyclohexane	
8	19.209	0.42	Heneicosanoic acid, methyl ester	

9	20.138	0.50	1-Hexyl-2-nitrocyclohexane	
10	20.384	1.05	Ethanone, 1-(4-cyclohexylphenyl)-	
11	21.027	6.07	Pyridazin-3(2H)-one, 4,5-di(ethylthio)-2-phenyl-	
12	21.214	10.58	2H-Isoindole-2-acetic acid, 1,3-dihydro-1,3-dioxo-, ethyl ester	
13	21.378	13.17	2-[5-(2-Methyl-benzooxazol-7-yl)-1H-pyrazol-3-yl]-phenol	
14	23.114	3.90	3H-Pyrazol-3-one, 4-[[4-(diethylamino) phenyl]imino]-2,4-dihydro-5-methyl-2-phenyl-	
15	23.415	1.13	Benzamide, N-methyl-4-(4-methyl-1-phthalazinylamino)-	
16	25.172	0.64	Diazene, bis[2,6-bis(1-methylethyl)phenyl]-	

### 3.3 Antifungal activity and synergistic antifungal activity

*P. mentha* essential oil and *P. pinnata* essential oil and 2 polyene antibiotics viz. Amphotericin B and Nystatin and fourazole group of antibiotics viz. Ketoconazole, Fluconazole, Clotrimazole and Itraconazole were evaluated for their antifungal activity against 19 clinical isolates of *Candida*. The antifungal activity of essential oils alone, antibiotics alone and essential oils plus antibiotics i.e. synergistic antifungal activity was also evaluated.

#### Amphotericin B

The antifungal activity of essential oils of *P. mentha* and *P. pinnata*, antibiotic Amphotericin B (AMP) and its synergistic activity i.e. essential oils plus Amphotericin B against all the 19 clinical *Candida* isolates is given in Fig. 3A and their mean zone of inhibition is given in Fig. 3B. Increase in fold area (IFA) values are given in Table 3. The antibiotic AMP alone showed antifungal activity with mean zone of inhibition 14.26 mm (Fig. 3B). The inhibition zone of AMP ranged from 12 mm to 19 mm and AMP alone exhibited maximum zone of inhibition against isolate C42 (19 mm) (Fig. 3A).

The *P. mentha* oil alone showed antifungal activity against 10 clinical *Candida* isolates. The zone of inhibition ranged from 9.5 mm to 10.5 mm. Maximum zone of inhibition of *P.*

*mentha* essential oil alone was against isolates C13 (10.5 mm) (Fig. 3A). AMP plus *P. mentha* essential oil showed antifungal activity against all the isolates except against C30 (Fig. 3A). The inhibition zone ranged from 9.5 mm to 13.5 mm. The synergistic antifungal activity was against 2 isolates i.e. C12 and C43; their IFA values were 2.06 and 0.08 respectively (Fig 3A) (Table 3). The *P. pinnata* oil alone did not show antifungal activity against any of the clinical *Candida* isolates (Fig. 3A). AMP plus *P. pinnata* oil showed antifungal activity against only 3 isolates C1, C3 and C6 (Fig. 3A). AMP plus *P. pinnata* oil did not show any synergistic antifungal activity against any clinical *Candida* isolates (Fig 3A).

#### Nystatin

The antifungal activity of essential oils of *P. mentha* and *P. pinnata*, antibiotic Nystatin (NYS) and its synergistic activity i.e. essential oils plus Nystatin against all 19 clinical *Candida* isolates is given in Fig. 4A and their mean zone of inhibition is given in Fig. 4B. Increase in fold area (IFA) values are given in Table 3.

The antibiotic NYS alone showed antifungal activity with mean zone of inhibition 18.56 mm (Fig. 4B). The inhibition zone of NYS alone ranged from 16 mm to 23 mm and NYS

alone exhibited maximum zone of inhibition against isolates C21, C41 and C42 (23 mm) (Fig. 4A). The essential oil *P. mentha* alone showed antifungal activity against 10 clinical *Candida* isolates (Fig. 4A). The inhibition zone ranged from 9.5 mm to 10.5 mm. Maximum zone of inhibition of *P. mentha* oil alone was against isolate C13 (10.5 mm) (Fig. 4A). NYS plus *P. mentha* oil showed antifungal activity against all the 19 isolates (Fig. 4A). The inhibition zone ranged from 15 mm to 23.5 mm. Maximum zone of inhibition was against isolates C6 and C41 (23.5 mm) (Fig. 4A). The synergistic antifungal activity was against 8 isolates i.e. C1, C2, C3, C6, C12, C15, C26 and C41 (Fig. 4A). The IFA values ranged from 0.04 to 6.11; Maximum IFA value was against isolate C12 (6.11) (Table 3). The *P. pinnata* oil alone did not show antifungal activity against any of the clinical *Candida* isolates (Fig. 4A). NYS plus *P. pinnata* essential oil showed antifungal activity against all the 19 isolates (Fig. 4A). The inhibition zone ranged from 14 mm to 20.5 mm and maximum zone of inhibition was against isolate C12 (20.5 mm). The synergistic antifungal activity was against 2 isolates i.e. C12 and C26 (Fig. 4A) and their IFA values were 10.67 and 0.06 respectively (Table 3).

### Ketoconazole

The antifungal activity of essential oils of *P. mentha* and *P. pinnata*, antibiotic Ketoconazole (KT) and its synergistic activity i.e. essential oils plus Ketoconazole against all 19 clinical *Candida* isolates is given in Fig. 5A and their mean zone of inhibition is given in Fig. 5B. Increase in fold area (IFA) values are given in Table 3.

The antibiotic KT alone showed antifungal activity with mean zone of inhibition 7.58 mm (Fig. 5B). The antibiotic KT alone showed antifungal activity only against isolates C30 and C41 and their inhibition zones were 16 mm and 26 mm respectively (Fig. 5A). The *P. mentha* essential oil alone showed antifungal activity against 10 clinical *Candida* isolates, zone of inhibition ranged from 9.5 mm to 10.5 mm. Maximum zone of inhibition of *P. mentha* essential oil alone was against isolate C13 (10.5 mm) (Fig. 5A). KT plus *P. mentha* oil showed antifungal activity against all the 19 clinical *Candida* isolates (Fig. 5A). The inhibition zone ranged from 13.5 mm to 20.5 mm. Maximum zone of inhibition was against isolate C41 (20.5 mm) (Fig. 5A). The synergistic antifungal activity was against 17 isolates except against isolates C30 and C41 (Fig. 5A). The IFA values ranged from 4.06 to 6.56; Maximum IFA value was against isolates C12 and C18 (6.56) (Table 3). The *P. pinnata* oil alone did not show antifungal activity against any of the clinical isolates of *Candida* (Fig. 5A). KT plus *P. pinnata* oil showed antifungal activity against all the 19 isolates except against C44 (Fig. 5A). The inhibition zone ranged from 10 mm to 18.5 mm and maximum zone of inhibition was against isolate C41 (18.5 mm). The synergistic antifungal activity was against 16 isolates i.e. C1, C2, C3, C5, C6, C12, C13, C14, C15, C18, C21, C22, C23, C26, C42 and C43 (Fig. 5A). The IFA values ranged from 1.78 to 7.02; Maximum IFA value was against isolate C26 (7.02) (Table 3).

### Fluconazole

The antifungal activity of essential oils of *P. mentha* and *P. pinnata*, antibiotic Fluconazole (FLC) and its synergistic activity i.e. essential oils plus Fluconazole against all 19 clinical *Candida* isolates is given in Fig. 6A and their mean zone of inhibition is given in Fig. 6B. Increase in fold area (IFA) values are given in Table 3.

The antibiotic FLC alone showed antifungal activity with mean zone of inhibition 7.10 mm (Fig. 6B). FLC alone exhibited zone of inhibition against only 1 isolate C41 (27 mm) (Fig. 6A). The *P. mentha* oil alone showed antifungal activity against 10 clinical *Candida* isolates, zone of inhibition ranged from 9.5 mm to 10.5 mm. Maximum zone of inhibition of *Piper mentha* oil alone was against isolate C13 (10.5 mm) (Fig. 6A). FLC plus *P. mentha* oil showed antifungal activity against 17 clinical *Candida* isolates (Fig. 6A). The inhibition zone ranged from 11.5 mm to 14.5 mm. Maximum zone of inhibition was against isolate C15 (14.5 mm) (Fig. 6A). The synergistic antifungal activity was against 16 isolates i.e. C1, C2, C3, C5, C6, C12, C13, C14, C15, C21, C22, C23, C26, C42, C43 and C44 (Fig. 6A). The IFA values ranged from 2.67 to 4.84; Maximum IFA value was against isolate C15 (4.84) (Table 3). The *P. pinnata* oil alone did not show antifungal activity against any of the 19 clinical isolates of *Candida*, FLC plus *P. pinnata* oil showed antifungal activity against only 2 isolates; C3 and C41 (Fig. 6A); their inhibition zones were 12 mm and 16.5 mm respectively. The synergistic antifungal activity was against 1 isolate i.e. C3 and its IFA value was 3.0 (Fig. 6A) (Table 3).

### Clotrimazole

The antifungal activity of essential oils of *P. mentha* and *P. pinnata*, antibiotic Clotrimazole (CC) and its synergistic activity i.e. essential oils plus Clotrimazole against all 19 clinical *Candida* isolates is given in Fig. 7A and their mean zone of inhibition is given in Fig. 7B. Increase in fold area (IFA) values are given in Table 3.

The antibiotic CC alone showed antifungal activity with mean zone of inhibition 7.58 mm (Fig. 7B). The inhibition zone of CC alone ranged from 12 mm to 22 mm and CC alone exhibited maximum zone of inhibition against isolate C41 (22 mm) (Fig. 7A), while the essential oil *P. mentha* alone showed antifungal activity against 10 clinical *Candida* isolates (Fig. 7A). The inhibition zone ranged from 9.5 mm to 10.5 mm; maximum zone of inhibition was against isolate C13 (10.5 mm) (Fig. 7A). CC plus *P. mentha* oil showed antifungal activity against all the 19 isolates (Fig. 7A). The inhibition zone ranged from 10.5 mm to 15 mm. Maximum zone of inhibition was against isolate C41 (15 mm) (Fig. 7A). The synergistic antifungal activity was against 16 isolates i.e. C1, C2, C3, C5, C6, C12, C13, C14, C15, C18, C22, C23, C26, C42, C43 and C44 (Fig. 7A). The IFA values ranged from 2.25 to 4.06; maximum IFA value was against isolate C2 (4.06) (Table 3). The *P. pinnata* oil alone did not show antifungal activity against any of the clinical *Candida* isolates. CC plus *P. pinnata* oil showed antifungal activity against only 2 isolates C30 and C41; their inhibition zone was 12 mm and 10.5 mm respectively (Fig. 7A). CC plus *P. pinnata* oil did not show any synergistic antifungal activity against any of clinical isolates of *Candida* (Fig. 7A).

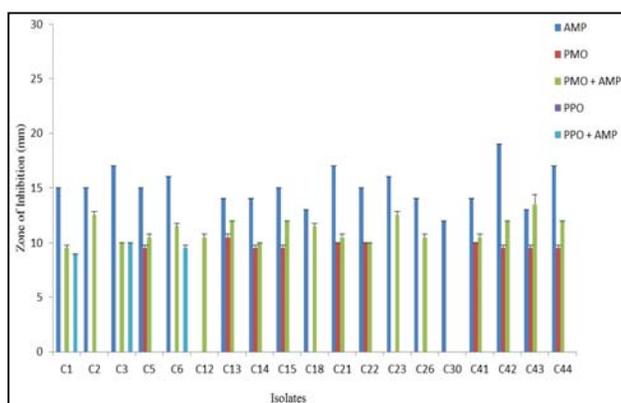
### Itraconazole

The antifungal activity of essential oils of *P. mentha* and *P. pinnata*, antibiotic Itraconazole (IT) and its synergistic activity i.e. essential oils plus Itraconazole against all 19 clinical *Candida* isolates is given in Fig. 8A and their mean zone of inhibition is given in Fig. 8B. Increase in fold area (IFA) values is given in Table 3.

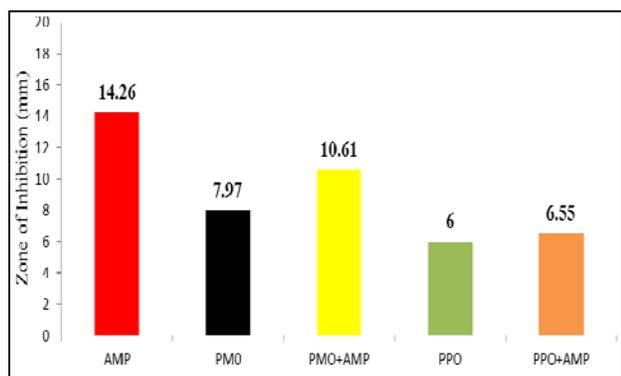
The antibiotic IT alone showed antifungal activity against 2 isolates C30 and C41, with mean zone of inhibition 6.74 mm (Fig. 8B). The inhibition zone of IT ranged from 12 mm to 14 mm and IT alone exhibited maximum zone of inhibition

against isolate C41 (14 mm) (Fig. 8A). The essential oil *P. mentha* alone showed antifungal activity against 10 clinical *Candida* isolates (Fig. 8A). The inhibition zone ranged from 9.5 mm to 10.5 mm. Maximum zone of inhibition was against isolate C13 (10.5 mm) (Fig. 8A). IT plus *P. mentha* oil showed antifungal activity against 18 isolates (Fig. 8A). The inhibition zone ranged from 10.5 mm to 14 mm. Maximum zone of inhibition was against isolate C41 (14 mm) (Fig. 8A). The synergistic antifungal activity was against 16 isolates i.e. C1, C2, C3, C5, C6, C12, C14, C15, C21, C22, C23, C26, C30, C43 and C44 (Fig. 8A). The IFA values ranged from 0.08 to 3.69; maximum IFA value was against isolate C2 and C44 (3.69) (Table 3). The *P. pinnata* oil alone did not show antifungal activity against any of the clinical *Candida* isolates. IT plus *P. pinnata* oil showed antifungal activity against only 3 isolates (Fig. 8A). The inhibition zone ranged from 10 mm to 13 mm and maximum zone of inhibition against isolate C3 (13 mm) (Fig. 8A). The synergistic antifungal activity was against only 1 isolate i.e. C30; its IFA value was (0.08) (Fig. 8A) (Table 3).

### Amphotericin B

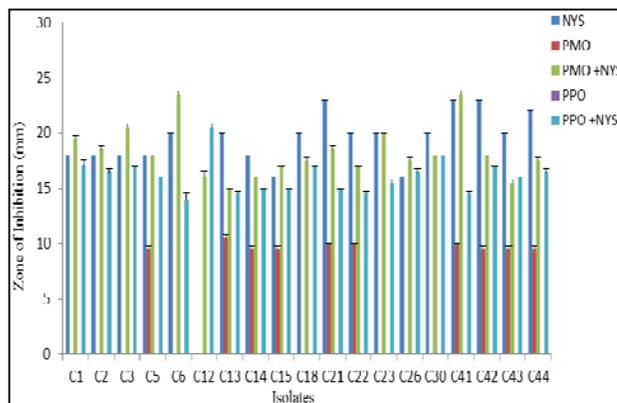


**Fig 3A:** Synergistic antifungal activity of *Piper mentha* essential oil (PMO) and *Pongamia pinnata* essential oil (PPO) with Amphotericin B (AMP) antibiotic against 19 multidrug resistant *Candida* isolates

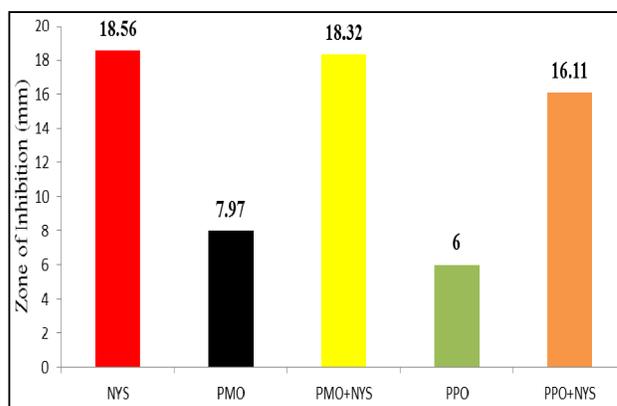


**Fig 3B:** Mean values of zone of inhibition of Amphotericin B (AMP) alone and *Piper mentha* essential oil (PMO) and *Pongamia pinnata* essential oil (PPO) and antibiotic in combination against 19 multidrug resistant *Candida* isolates

### Nystatin

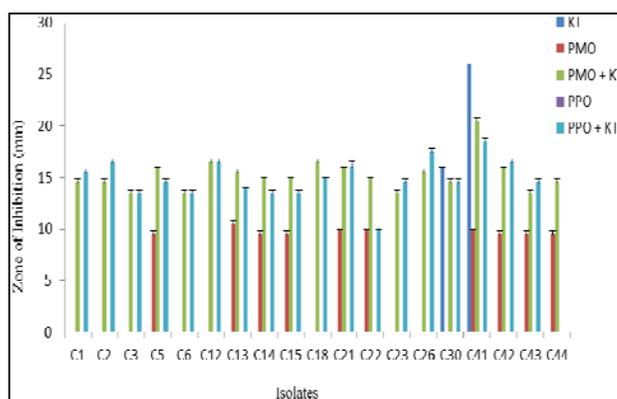


**Fig 4A:** Synergistic antifungal activity of *Piper mentha* essential oil (PMO) and *Pongamia pinnata* essential oil (PPO) with Nystatin (NYS) antibiotic against 19 multidrug resistant *Candida* isolates

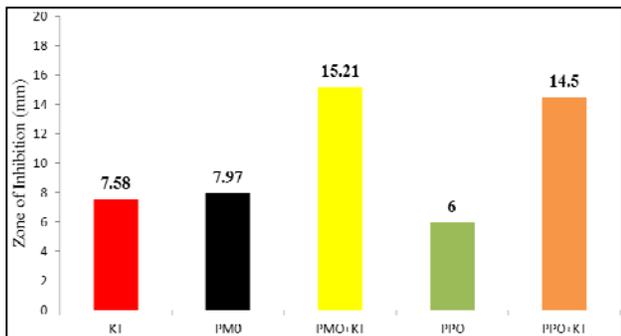


**Fig 4B:** Mean values of zone of inhibition of Nystatin (NYS) alone and *Piper mentha* essential oil (PMO) and *Pongamia pinnata* essential oil (PPO) and antibiotic in combination against 19 multidrug resistant *Candida* isolates

### Ketoconazole

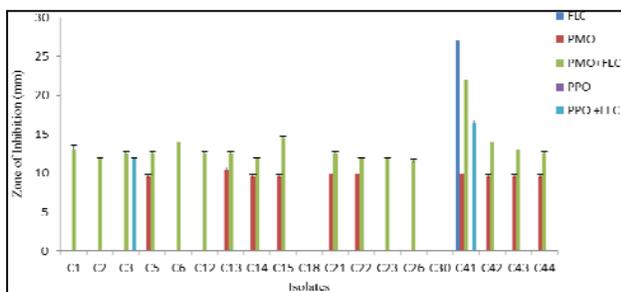


**Fig 5A:** Synergistic antifungal activity of *Piper mentha* essential oil (PMO) and *Pongamia pinnata* essential oil (PPO) with Ketoconazole (KT) antibiotic against 19 multidrug resistant *Candida* isolates

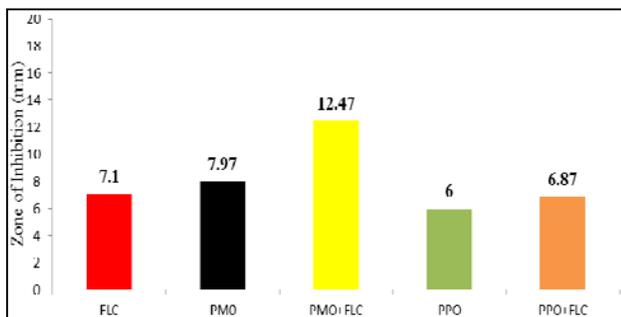


**Fig 5B:** Mean values of zone of inhibition of Ketoconazole (KT) alone and *Piper mentha* essential oil (PMO) and *Pongamia pinnata* essential oil (PPO) and antibiotic in combination against 19 multidrug resistant *Candida* isolates

**Fluconazole**

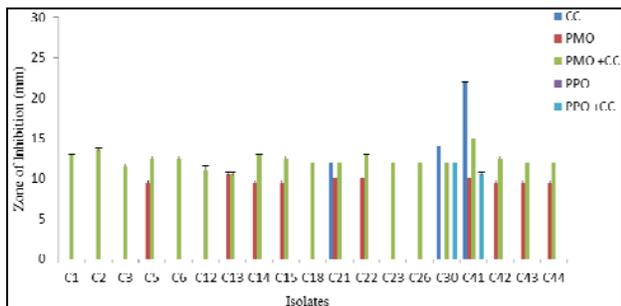


**Fig 6A:** Synergistic antifungal activity of *Piper mentha* essential oil (PMO) and *Pongamia pinnata* essential oil (PPO) with Fluconazole (FLC) antibiotic against 19 multidrug resistant *Candida* isolates

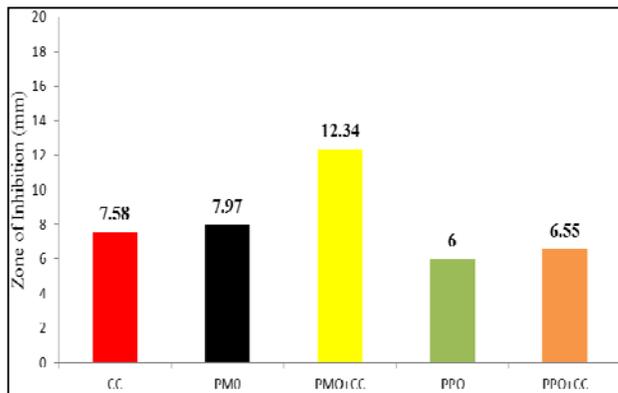


**Fig 6B:** Mean values of zone of inhibition of Fluconazole (FLC) alone and *Piper mentha* essential oil (PMO) and *Pongamia pinnata* essential oil (PPO) and antibiotic in combination against 19 multidrug resistant *Candida* isolates

**Clotrimazole**

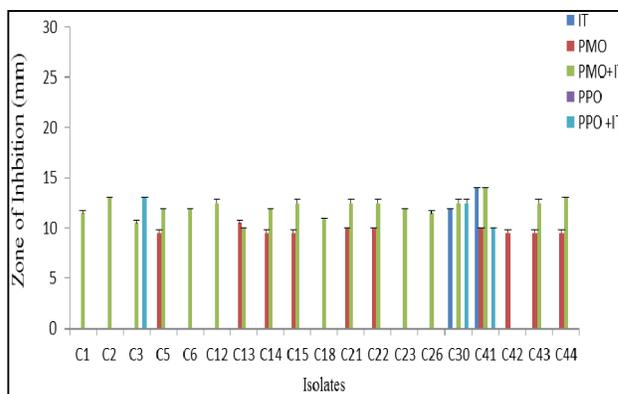


**Fig 7A:** Synergistic antifungal activity of *Piper mentha* essential oil (PMO) and *Pongamia pinnata* essential oil (PPO) with Clotrimazole (CC) antibiotic against 19 multidrug resistant *Candida* isolates

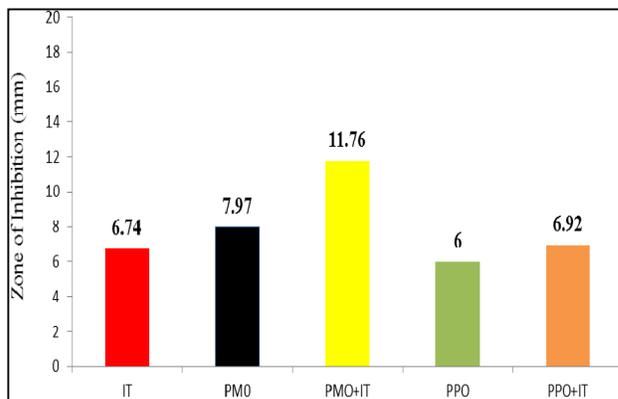


**Fig 7B:** Mean values of zone of inhibition of Clotrimazole (CC) alone and *Piper mentha* essential oil (PMO) and *Pongamia pinnata* essential oil (PPO) and antibiotic in combination against 19 multidrug resistant *Candida* isolates

**Itraconazole**



**Fig 8A:** Synergistic antifungal activity of *Piper mentha* oil (PMO) and *Pongamia pinnata* essential oil (PPO) with Itraconazole (IT) antibiotic against 19 multidrug resistant *Candida* isolates essential



**Fig 8B:** Mean values of zone of inhibition of Itraconazole (IT) alone and *Piper mentha* essential oil (PMO) and *Pongamia pinnata* essential oil (PPO) and antibiotic in combination against 19 multidrug resistant *Candida* isolates

**Table 3:** Increase in fold area (IFA) of six different antibiotics with *Piper mentha* and *Pongamia pinnata* essential oils

Isolates No.	IFA values of <i>Piper mentha</i> oil plus antibiotics						IFA values of <i>Pongamia pinnata</i> oil plus antibiotics					
	AMP	NYS	FLC	KT	CC	IT	AMP	NYS	FLC	KT	CC	IT
C1	-	0.17	3.69	4.84	3.69	2.67	-	-	-	5.67	-	-
C2	-	0.17	3	4.84	4.06	3.69	-	-	-	6.56	-	-
C3	-	0.30	3.34	4.06	2.67	2.06	-	-	3	4.06	-	-
C5	-	-	3.34	6.11	3.34	3	-	-	-	4.84	-	-
C6	-	0.38	4.44	4.06	3.34	3	-	-	-	4.06	-	-
C12	2.06	6.11	3.34	6.56	2.36	3.34	-	10.67	-	6.56	-	-
C13	-	-	3.34	4.44	2.25	-	-	-	-	5.67	-	-
C14	-	-	3	6.11	3.69	3	-	-	-	4.06	-	-
C15	-	0.13	4.84	5.25	3.34	3.34	-	-	-	4.06	-	-
C18	-	-	-	6.56	3	2.44	-	-	-	5.25	-	-
C21	-	-	3.34	6.11	-	3.34	-	-	-	6.11	-	-
C22	-	-	3	5.25	3.69	3.34	-	-	-	1.78	-	-
C23	-	-	3	4.06	3	3	-	-	-	4.84	-	-
C26	-	0.20	2.67	5.67	3	2.67	-	0.06	-	7.02	-	-
C30	-	-	-	-	-	0.08	-	-	-	-	-	0.08
C41	-	0.04	-	-	-	-	-	-	-	-	-	-
C42	-	-	4.44	6.11	3.34	-	-	-	-	6.56	-	-
C43	0.08	-	3.69	4.06	3	3.34	-	-	-	4.84	-	-
C44	-	-	3.34	4.84	3	3.69	-	-	-	-	-	-

#### 4. Discussion

Essential oils are the volatile liquids of the secondary metabolism of aromatic plants. All plant organs like seeds, barks, fruits, peels, leaves, stems, etc are capable of synthesizing essential oils and are present in cavities, canals, epidermal cells or glandular trichomes [31]. They are widely distributed in plant kingdom especially in families like Lauraceae, Meliaceae, Myrtaceae, Rutaceae, Fabaceae, Lamiaceae, Asteraceae, Aristolochiaceae, Cupressaceae, etc [32].

In the present work, the GC-MS analysis of *P. mentha* oil revealed the presence of various compounds like Propylene Glycol, D-Limonene, Benzyl alcohol, p- Menthone, Menthol, Cyclohexanol, beta- Elemene, Naphthalene, Cadinene. The main constituents in the essential oils of *M. piperita* were menthol, L-menthone and isomenthone; followed by neoisomenthol, pulegone and menthyl acetate [33]. Sokovic *et al.*, [34] identified 26 components in the essential oil of *M. piperita* but the main components were menthol, menthyl acetate and menthone. *M. piperita* cultivated in Italy showed the presence of menthol, menthyl acetate and menthofuran [35]. According to Yadegarina *et al.*, [36] the main components of essential oil of *M. piperita* from east Iran were  $\alpha$ -terpinene, isomenthone, trans-carveol, piperitenone oxide and  $\beta$ -caryophyllene. This variation in the components of essential oil of *M. piperita* is because of the influence of phenological status and environmental conditions which greatly influences the biosynthesis of essential oils of the plants [37]. The essential oils of *M. piperita* showed very good antifungal potential against the plant pathogenic fungi [33]; antibacterial, antifungal and antioxidant activities [38]; antimicrobial and antioxidant activity [39] which is due to the main components of the essential oils viz. menthol, menthone and carvone. There are many reports in the literature that combination of essential oils generally exhibit better antimicrobial activity than individual components and they play complementary role with each other. This synergistic effect is because of the components of the essential oil [40, 41].

The GC-MS analysis of *P. pinnata* oil revealed the presence of various compounds like Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, 9-Octadecenoic acid, methyl ester, (E), Methyl stearate, Oleic Acid, Octadecanoic acid, 1-Hexyl-2-nitrocyclohexane, Heneicosanoic acid, methyl ester, 1-Hexyl-

2-nitrocyclohexane, Ethanone, 1-(4-cyclohexylphenyl)-, Pyridazin-3(2H)-one, 4,5-di(ethylthio)-2-phenyl-, 2H-Isoindole-2-acetic acid, 1,3-dihydro-1,3-dioxo-, ethyl ester, 2-[5-(2-Methyl-benzooxazol-7-yl)-1H-pyrazol-3-yl]-phenol, 3H-Pyrazol-3-one, 4-[[4-(diethylamino) phenyl]imino]-2,4-dihydro-5-methyl-2-phenyl-, Benzamide, N-methyl-4-(4-methyl-1-phthalazinylamino)-, Diazene, bis[2,6-bis(1-methylethyl)phenyl]. Wagh *et al.*, [42] reported antibacterial and antifungal activity of essential oil of *P. pinnata*. The seed oil contains karanjin, a bioactive molecule with many important biological activities [43].

The most currently used antifungal antibiotic is Flucanazole but HIV positive patients have developed resistance to this antibiotic; and in some cases this triggers resistance to other azole antibiotics and also lesser pathogens than *C. albicans* like *C. glabarata* and *C. krusei* also develop resistance [44]. Therefore there is an urgent need to develop alternate antifungals and plant essential oils are one of the most promising sources either alone or in combination. The two major components of plant essential oils are terpenoids and phenols which are lipophilic in nature and are responsible for their antimicrobial activity. They induce structural and functional damage in the microorganisms. The essential oils disrupt membrane permeability and osmotic balance of the cell. The transport processes are disrupted, membrane proteins and other components of the cell are destroyed [45]. Thus they show good antimicrobial property [46, 47]. Bouhdid *et al.* [48] reported that *Origanum compactum* essential oil retard the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* by altering membrane potential and permeability. Ferreira *et al.*, [49] reported antifungal activity of *Curcuma longa* essential oil. Djihane *et al.*, [50] reported the antibacterial and antifungal activity of essential oil of aerial parts of *Helichrysum italicum*. *Artemisia sieberi* and *Origanum vulgare* essential oils effectively showed antifungal activity against clinical isolates of *Candida* [51]. Antifungal activity of Thymus oil is reported by Pina-Vaz *et al.*, [52]. Pozzatti *et al.*, [53] reported antifungal activity of spice essential oils against fluconazole-resistant and fluconazole-susceptible *Candida* spp.

#### 5. Conclusion

In the present work, amongst both the essential oils, *P.*

*mentha* essential oil showed better synergistic antifungal activity than *P. pinnata* essential oil with all the six antibiotics used. The best synergistic activity was shown with Ketoconazole, an azole antibiotic. It can be stated that combination therapy between plant essential oil and antibiotics is one of the most promising approach to treat multi drug resistant *Candida* species. Similar results are also reported by Adrar *et al.*,<sup>[54]</sup>. The essential oil of *P. mentha* in combination with antibiotics could be used as a natural antimicrobial agent against *Candida*.

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