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Antibacterial and Anticandidal Activities of Common Essential Oil Constituents

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Abstract: Essential oils and some of their oxygenated constituents are known to possess antimicrobial activity. In the last 30 years, there is a dramatic increase in the number of resistant microorganisms against available antimicrobials and a tendency towards natural products; consequently, scientists have been forced to discover new bioactive agents preferably from nature. As a result of this, so many antimicrobial screening works have been published on plant essential oils including miscellaneous screening methods and several microorganism strains. The aim of this study was to determine the MIC values of 65 monoterpenoids and 3 phenyl propanoids commonly found in essential oils, against 24 pathogenic bacteria and *Candida* strains, by using standard reference broth dilution methods (CLSI M7-A7 and M27-A2). According to broth microdilution test results, when compared with standard agents, monoterpene hydrocarbons generally showed weak antibacterial effects (>16 to 4 mg/mL) where the oxygenated monoterpenes inhibited the microbial growth between the concentrations of 16 to 0,03 mg/mL. Generally, tested compounds demonstrated better inhibitory effects on *Candida* strains then the bacteria panel. The most effective microbial growth inhibitor constituents were determined as carvacrol, thymol, cumin alcohol, terpinen-4-ol, α-terpineol, lavandulol, estragol and thymoquinone.

Keywords: Monoterpenoids; MIC; essential oil; antibacterial; anticandidal; CLSI.

1. Introduction

Terpenoids constitute the largest group of natural compounds with more than 30,000 members [1]. These molecules play important roles during the life of plants, such as growth, development, reproduction and defence [2]. Terpenes are hydrocarbons resulting from the integration of several isoprene units (C5). They are classified, by the chain of isoprene units, as hemiterpenes, monoterpenes, sesquiterpenes, sesterterpenes, diterpenes, triterpenes, tetraterpenes and polyterpenes [1-3]. Terpenoids are synthesized in plant cells by isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP). There are two well-known metabolic pathways generating terpenoids: named mevalonic acid and the non-mevalonate (MEP/DOXP) [4, 5].

Monoterpenes comprise two C5 units that are linked in head to tail manner. Approximately, 1500 monoterpenes and their oxygenated derivatives (monoterpenoids) are documented. In nature,

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monoterpenes occur in plant essential oils mainly as hydrocarbons and their oxygenated derivatives such as alcohols, ethers, aldehydes, ketones, carboxylic acids and esters [1, 6].

Especially oxygenated compounds in the essential oils are responsible for the biological activity and generally attract more attention [7, 8].

Essential oils (also called volatile or ethereal oils) are complex mixtures of volatile compounds produced by living organisms and generally isolated by water distillation from a whole plant or individual parts [6]. Naturally occurring mono- and sesquiterpenes, benzenoids, phenyl propanoids and some fatty acids are major compounds of essential oils, which demonstrate several biological activities individually or as mixtures [9, 10]. Essential oil bearing plants have received much more attention as natural sources for flavour, fragrance, food and pharmaceutical industries, because of their remarkable aromatic and antimicrobial properties [11-14]. Furthermore, during the past decades, essential oils and some of their constituents have been confirmed as analgesic, anti-inflammatory, antiviral, anticancer, insect repellent, antioxidant and potential enhancer agent for transdermal drug delivery [11, 15, 16].

Some of the essential oils are known to possess remarkable antibacterial and antifungal properties because of several constituents in the oil, which are already tested in this study. Cinnamon, rosemary [7] peppermint, oregano, tea tree, thyme, basil, clove, eucalyptus, lavender, sage, fennel, coriander, anise, lemongrass and savory essential oils are the most studied and possessed inhibitory effects against human, animal and/or plant pathogen microorgansms *in vitro* and *in vivo* [12, 13, 17-26].

In the last 30 years, the development of microbial resistance to antibiotics and paucity of discovery of new antibiotics in human and animal therapies have stimulated scientists to find new natural antimicrobials. As a result of this, great number of bioactivity screening works have been published about antimicrobial evaluation of essential oils and their constituents. When “essential oil” and “antimicrobial” keywords entered on the “Web of Science Search”, it resulted in approximately 5000 scientific papers; moreover, this number is 45.000 at “Google Scholar Search” (between the years of 1987 to 2017). There exist a number of tests in those studies for evaluating the inhibitory potency of essential oils and their constituents such as diffusion, dilution and bioautographic methods. The diffusion methods except for E-Test are generally considered as semi-qualitative techniques, since they only give us “inhibition zone diameter (mm or inches)” and an idea about the compound as “active” or “inactive”. They do not give us the exact inhibitory concentration of the compounds. However, by using agar dilution or macro- and microdilution techniques one can determine minimal inhibitory or microbicidal concentrations (MIC, MBC, IC50, IC90 etc.) of the test compounds against selected microorganisms [18, 21]. Test microorganisms panel is generally composed of standard reference collections (ATCC, NRRL, NCTC, JCM, etc.) and/or clinically isolated strains of pathogenic resistant bacteria and fungi species [27-32].

Papers about antimicrobial activity generally give MIC result of a natural compound against similar microorganisms. These minimum inhibitory concentration (MIC) results, even the same microorganism strains used, are usually varied from work to work. The different or instable test protocols, false strains, contaminated microorganisms, quality of laboratory facilities and the ability of personnel may explain the variability of the results.

In the present study, by using mostly recommended standard CLSI reference micro-dilution broth methods and standard microorganism strains, uniform and repeatable MIC results of the common 65 monoterpenoids (including some stereo-isomers), 3 phenylpropanoids (estragol, *trans*-anethol and eugenol) that are widely found in essential oils were determined against 24 different pathogenic bacteria and *Candida* strains [33-34]. With this study, most of the commercially available authentic monoterpenoids and if available, their isomers were tested together with a validated method for the first time here. To the best of our knowledge, there is a few works about antimicrobial activities of different stereoisomers of the monoterpenes. Furthermore, the study will be a useful comparison guide for the further bioactivity works on essential oils that are rich in monoterpenoids.

2. Materials and Methods

2.1. Essential Oil Constituents

At the present study, commercially available authentic samples of monoterpene hydrocarbons, oxygenated monoterpenes and some phenylpropanoids estragol, *trans*-anethol and eugenol were used as test compounds (Table 1).

Table 1. Tested essential oil constituents

Hydrocarbons	[4](+)-Neomenthol, ≥95% (A)	(-)-Menthone, 90% (A)
p-Cymene, 99% (A)	(-)-Menthol, 98.5% (A)	(+)-Menthone, ≥98.5% (F)
[1]Ocimene, >90% (A)	[6](+)-Menthol, 99% (A)	(*R*)-(+)-Pulegone, 97% (A)
Myrcene, ≥95.0% (F)	[2](+)-Isopulegol, 99% (A)	(*S*)-(-)-Pulegone, 98% (A)
(*R*)-(+)-Limonene, 99.0% (F)	[2](-)-Isopulegol, 99% (A)	(*R*)-(-)-Carvone, 98% (A)
(*S*)-(-)-Limonene, ≥99.0% (F)	(*R*)-(+)-*α*-Terpineol, 97.0% (F)	(*S*)-(+)-Carvone, ≥97% (A)
Terpinolene, ≥90% (A)	(*S*)-(−)-*α*-Terpineol, 97.0% (F)	(1*S*)-(-)-Verbenone, 94% (A)
γ-Terpinene, ≥98.5% (F)	[2](-)-Terpinen-4-ol, 95% (A)	(±)-Camphor, EP Ref Std (F)
(+)-3-Carene, ≥98.5% (F)	[2](+)-Terpinen-4-ol, ≥98.5% (F)	(1*R*)-(+)-Camphor, 98% (A)
Sabinene, natural, 75% (A)	[2](-)-Carveol, ≥98.5% (F)	(1*S*)-(-)-Camphor, %99 (A)
(1*R*)-(+)-*α*-Pinene, 97% (A)	Cumin alcohol, ≥97% (A)	**Esters**
(1*S*)-(-)-*α*-Pinene, 97% (A)	(1*R*)-(-)-Myrtenol, 95% (A)	Geranyl acetate, ≥97% (A)
[2](+)-*β*-Pinene, ≥98.5% (F)	(*S*)-*cis*-Verbenol, 95% (A)	(1*R*)-(-)-Menthyl acetate, ≥98.5% (A)
[2](-)-*β*-Pinene, ≥98.5% (F)	(+)-Borneol, 97.5% (F)	[2](-)-Bornyl acetate, 95% (A)
[2]*α*-Pinene, 98.5% (A)	(-)-Borneol, 99% (A)	[2](+)-Bornyl acetate, ≥98.5% (F)
(+)-Camphene, ≥80% (A)	Isoborneol, 95% (A)	Isobornyl acetate, ≥95% (A)
(-)-Camphene, ≥70% (A)	Lavandulol, ≥95% (F)	Linalyl acetate, ≥97% (A)
(*R*)-(-)*α*-Phellandrene, ≥95% (A)	Carvacrol, %99 (A)	**Others**
Alcohols	Thymol, ≥99.0% (S)	Estragol, 99% (F)
[2](-)-Linalool, ≥95% (A)	**Aldehydes and Ketones**	*Trans*-Anethol, 99% (A)
Geraniol, 98% (A)	(*R*)-(+)-Citronellal, 90% (A)	Eugenol, ≥98% (A)
Nerol, ≥97.0% (F)	(*S*)-(-)-Citronellal, 96% (A)	Thymoquinone, 99% (A)
(*S*)-(-)-*β*-Citronellol, ≥99% (A)	(1*R*)-(-)-Myrtenal, 98% (A)	1,8-cineole, 99% (A)
(*R*)-(+)-*β*-Citronellol, 98% (A)	Citral, ≥96% (A)	
[3](+)-Isomenthol, ≥95% (Fluka)	Cuminaldehyde, 98% (A)	

(A): ALDRICH, (F): FLUKA, (S): SIGMA, [1]mixture of isomers, [2]sum of enantiomers, [3](1*S*,2*R*,5*R*), [4](1*S*,2*S*,5*R*), [5](1*R*,2*S*,5*R*), [6](1*S*,2*R*,5*S*)

2.2. Antimicrobial susceptibility test

Antibacterial and anticandidal effects of the compounds were screened by using partly modified CLSI (formerly NCCLS) micro dilution broth methods M7-A7 (Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically) and M27-A2 (Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts), respectively [33-34]. Unlike the protocol, stock solutions of the test samples were prepared at the concentrations of 32 mg/mL where the standard agents were prepared in accordance with CLSI methods.

Candida cultures were inoculated from the -85 °C onto potato dextrose agar (Fluka) while bacteria were inoculated onto Mueller Hinton agar (Fluka) for checking purity and viability. All microdilution broth tests were performed by using sterile 96 U-shaped multi-well microdilution plates (Brand) in laminar flow cabinet. Antibacterial test results were read after the incubation period at 35±2 ºC, 16-20 h. The MIC data from anticandidal tests was obtained after 24 h and 48 h separately.

The visible growth in each plate was compared with that of the growth control well (drug-free) by using a reading mirror. A considerable decrease in turbidity was accepted as growth inhibition. Furthermore, according to M27-A2 method, *C. krusei* (ATCC® 6258) and *C. parapsilosis* (ATCC® 22019) were used as quality control strains. To precision and accuracy of the susceptibility tests procedure, the MIC results of the standard antimicrobial agents against QC strains were checked from the CLSI-MIC limits tables. All the experiments were performed in duplicate. Ampicillin (Sigma-Aldrich) and Chloramphenicol (Fluka) were used as antibacterial agents where the Amphotericin-B (Sigma-Aldrich) and Ketoconazole (Sigma-Aldrich) were antifungal references.

2.3. Microorganisms

Gram negatives; *Pseudomonas aeruginosa* (Pa, ATCC 27853), *Enterobacter aerogenes* (Ea, NRRL 3567), *Proteus vulgaris* (Pv, NRRL B-123), *Serratia marcescens* (Sm, NRRL B-2544), *E. coli* (Ec, ATCC 8739), *E. coli* O157:H7 (Ec^a, RSSK 234), *Salmonella typhimurium* (St, ATCC 14028), Gram positives; *Bacillus cereus* (Bc, NRRL B-3711), *Bacillus subtilis* (Bs, NRRL B-4378), *Staphylococcus aureus* (Sa, ATCC 43300), *Listeria monocytogenes* (Lm, ATCC 19111), *Staphylococcus epidermidis* (Se, ATCC 14990) were used as bacteria panel while *Candida albicans* (Ca^a; ATCC 10231 and Ca^b; ATCC 24433), *Candida utilis* (Cu; NRRL Y-900), *Candida krusei* (Ck^a; NRRL Y-7179 and Ck^b; ATCC 6258), *Candida zeylanoides* (Cz; NRRL Y-1774), *Candida glabrata* (Cg^a; ATCC 2001 and Cg^b; ATCC 66032), *Candida tropicalis* (Ct^a; ATCC 1369 and Ct^b; ATCC 750), *Candida parapsilosis* (Cp^a; NRRL Y- 12696 and Cp^b; ATCC 22019) were used as *Candida* test strains.

3. Results and Discussion

65 monoterpenoids some with stereo isomers and three phenylpropanoids were evaluated against pathogenic bacteria panel (7 Gram negative, 5 Gram positive) and *Candida* strains by CLSI standard antimicrobial susceptibility tests in comparison with commercial antimicrobial agents.

Tested compounds were represented in the tables as separate groups (monoterpene hydrocarbons, alcohols, aldehydes, ketones, esters, phenylpropanoids and other oxygenated monoterpenes). The obtained MIC results from all authentic constituents and standard antibacterial and antifungal agents are given in mg/mL. Antibacterial activities of the compounds were presented in Table 2 to 4.

According to broth microdilution test results, when compared with standard agents, monoterpene hydrocarbons generally showed weak antibacterial effects between the concentrations of 4.0 mg/mL to >16.0 mg/mL (over the concentration of stock solution). Previous studies generally reported that the monoterpene hydrocarbons were inactive compounds because of their limited water solubility [35]. However, using partly modified standardized microdilution protocols the solubility problem is solved thanks to using pure DMSO for the preparation of stock solutions and all dilution stages. On the other hand, due to the hydrophilic structure of the gram-negative outer membrane of forms a barrier against the highly hydrophobic molecules such as monoterpene hydrocarbons. In general the cytotoxicity of the volatile constituents against bacteria are mostly due to their hydroxyl, aldehyde and ketone groups [21].

Aldehydes, ketones and esters showed a variable degree of antibacterial activities between the concentrations of 0.25 to 16.0 mg/mL. *Bacillus subtilis* was strongly inhibited by citral, myrtenal and (*R*)-(+)-citronellal having MIC values of 0.5, 0.5 and 0.25 mg/mL, respectively, compared to aliphatic alcohols and monoterpene hydrocarbons. The most active compounds against bacteria strains determined as monoterpene alcohols and phenols, especially (+)-menthol, (+)-isomenthol, cumin alcohol, carvacrol and thymol (Table 3). On the other hand, minus isomers of α-pinene, limonene, camphene, terpineol, borneol, terpinen-4-ol and β-citronellol showed better inhibitory effects than their plus isomers. The minus isomer of limonene inhibited the growing of *E. coli*, *P. aeruginosa*, *B. cereus*, *B. subtilis*, *S. aureus* and *S. epidermidis* at the half dose of the plus isomer (Table 2). Similarly to our results a previous study reported that the minus isomer of the limonene showed two or three

times stronger inhibitory effects against *E. coli, S. aureus, K. pneumonia, Moraxella catarrhalis* and *Cryptococcus neoformans* than its plus isomer [36].

Eugenol and 1,8-cineole exhibited moderate inhibitory effects (0.5-8.0 mg/mL, MICs) while an oxygenated monoterpene and also a quinone derivative thymoquinone showed strong effects at the concentrations between 0.03 to 1 mg/mL.

The antibacterial tests results showed that Gram (+) *Bacillus cereus* and *Bacillus subtilis* more susceptible to the tested essential oil constituents than the bacteria of other species. A previous works reported that, because of the structure of Gram (+) cell wall, lipophilic molecules diffused into the cells easily and demonstrate some modifications on the cell wall and the cytoplasm. On the other hand, Gram (-) bacteria cell wall has a lipopolysaccharide (LPS) layer that is a barrier to the penetration of hydrophobic compounds [37, 38].

Tested phenolic compounds carvacrol and thymol that are well known as natural antimicrobials [23, 39-43] revealed stronger inhibitory effects against Gram (+) bacteria than the Gram (-) (Table 3.). According to literature data phenolics are more effective against Gram (+) cell wall because of the complexity of Gram (-) bacteria cell wall as supported our findings [38]. In previous studies, the antibacterial effects of the carvacrol and thymol were attributed to their ability to permeabilize and depolarize the cytoplasmic membrane [40, 43, 44]. Its well known that some aldehydes like citral form some complexes with membrane proteins and inhibited the cell wall synthesis [45].

A recent study about antimicrobial and biofilm eliminating properties of some monoterpenes were reported similar MIC results to our study for thymol (0,25 mg/mL) menthol (1mg/mL) and 1,8-cineol (4 mg/mL) [46].

The present results also showed that the monoterpene alcohols were more active than their acetate, keton or aldehyde derivatives [35]. Similarly Kotan et al. [10] reported that the monoterpene alcohols are most active compounds *in vitro* against bacteria species among the essential oil constituents.

Table 2. Antibacterial Activity of Monoterpene Hydrocarbons (MIC, **mg/mL**)

	Gram (-)						Gram (+)					
Compounds	**Pa**	**Ea**	**Pv**	**Sm**	**Ec**	**Ec[a]**	**St**	**Bc**	**Bs**	**Sa**	**Lm**	**Se**
p-Cymene	8	8	8	8	8	8	16	4	8	4	4	4
Ocimene	8	>16	8	>16	>16	8	>16	8	4	16	8	8
Myrcene	8	>16	8	16	8	>16	16	8	8	8	8	16
(*R*)-(+)-Limonene	8	>16	8	8	>16	8	>16	8	16	16	4	8
(*S*)-(-)-Limonene	4	>16	8	8	8	8	>16	4	8	8	4	4
Terpinolene	4	4	4	4	4	4	16	4	8	16	8	2
γ-Terpinene	8	16	8	8	>16	8	>16	16	16	16	8	8
(+)-3-Carene	8	>16	8	8	8	8	16	4	8	4	4	8
Sabinene	8	>16	>16	>16	>16	16	>16	>16	16	16	16	16
(1*R*)-(+)-α-Pinene	8	>16	16	8	8	4	8	8	8	8	4	8
(1*S*)-(-)-α-Pinene	8	>16	16	8	8	4	16	4	4	4	8	4
(+)-*β*-Pinene	8	>16	>16	>16	>16	8	16	8	8	8	4	4
(-)-*β*-Pinene	8	>16	>16	>16	>16	8	16	>16	4	8	8	8
α-Pinene	8	>16	16	>16	8	4	16	4	4	4	4	4
(+)-Camphene	8	>16	>16	8	>16	8	8	>16	4	16	4	16
(-)-Camphene	8	>16	16	8	>16	16	16	8	4	8	16	8
(*R*)-(-)-*α*-Phellandrene	8	>16	8	>16	>16	16	>16	>16	8	16	16	8
St-1	0.128	0.032	0.001	0.016	0.002	0.001	0.001	0.002	0.001	0.001	0.001	0.001
St-2	0.064	0.002	0.004	0.004	0.001	0.001	0.001	0.004	0.001	0.008	0.002	0.001

[1]: O157:H7, St-1: Ampicillin (Fluka), St-2: Chloramphenicol (Sigma), >16: out of the maximum test concentration, **Pa**: *Pseudomonas aeruginosa*, **Ea**: *Enterobacter aerogenes*, **Pv**: *Proteus vulgaris*, **Sm**: *Serratia marcescens*, **Ec**: *E. coli*, **Ec[a]**: *E. coli* O157:H7, **St**: *Salmonella typhimurium*, Gram positives; **Bc**: *Bacillus cereus*, **Bs**: *Bacillus subtilis*, **Sa**: *Staphylococcus aureus*, **Lm**: *Listeria monocytogenes*, **Se**: *Staphylococcus epidermidis*

Table 3. Antibacterial Activity of Monoterpene Alcohols (MIC, mg/mL)

	Gram (-)						Gram (+)					
Compounds	**Pa**	**Ea**	**Pv**	**Sm**	**Ec**	**Ec[a]**	**St**	**Bc**	**Bs**	**Sa**	**Lm**	**Se**
(-)-Linalool	8	4	8	4	8	4	8	8	4	8	8	8
Geraniol	2	4	2	2	4	2	2	2	2	1	4	4
Nerol	2	4	2	2	4	2	4	2	2	2	4	4
(*S*)-(-)-*β*-Citronellol	2	4	1	1	4	4	4	2	4	4	2	4
(*R*)-(+)-*β*-Citronellol	2	4	4	1	4	4	4	2	4	4	4	4
(+)-Isomenthol	1	1	0.5	0.5	1	1	1	1	1	0.5	1	2
(+)-Neomenthol	4	4	4	8	8	4	8	4	8	8	8	8
(-)-Menthol	1	2	1	1	2	2	1	1	2	2	1	2
(+)-Menthol	1	2	0.25	0.5	1	0.5	1	1	0.5	1	1	1
(+)-Isopulegol	2	2	4	2	4	2	4	2	4	4	4	2
(-)-Isopulegol	2	2	4	2	4	2	4	2	4	4	4	4
(*R*)-(+)-*α*-Terpineol	4	4	4	4	2	2	2	2	1	2	4	2
(*S*)-(−)-*α*-Terpineol	4	1	2	2	1	1	2	2	1	1	2	2
(-)-Terpinen-4-ol	8	4	4	4	4	1	4	4	2	4	2	4
(+)-Terpinen-4-ol	1	4	4	2	4	2	4	1	4	4	4	4
(-)-Carveol	2	2	2	2	2	2	2	2	4	2	2	4
Cumin alcohol	2	0.5	0.5	0.5	1	0.25	0.5	0.5	0.5	1	1	0.5
(1*R*)-(-)-Myrtenol	2	2	1	2	4	2	2	2	1	2	2	2
(*S*)-*cis*-Verbenol	2	2	1	1	2	1	2	1	1	1	1	2
(+)-Borneol	2	2	1	1	2	1	2	1	1	2	2	2
(-)-Borneol	2	1	1	1	2	1	2	1	1	1	1	1
(±)-Isoborneol	1	2	1	1	2	1	2	1	1	2	2	2
Lavandulol	2	4	4	4	4	2	4	4	4	4	4	4
Carvacrol	0.25	0.25	0.25	0.25	0.12	0.25	0.12	0.06	0.06	0.12	0.12	0.12
Thymol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.12	0.12	0.12	0.5	0.5
St-1	0.128	0.032	0.001	0.016	0.002	0.001	0.001	0.002	0.001	0.001	0.001	0.001
St-2	0.064	0.002	0.004	0.004	0.001	0.001	0.001	0.004	0.001	0.008	0.002	0.001

[1]: O157:H7; St-1: Ampicillin, St-2: Chloramphenicol, **Pa**: *Pseudomonas aeruginosa*, **Ea**: *Enterobacter aerogenes*, **Pv**: *Proteus vulgaris*, **Sm**: *Serratia marcescens*, **Ec**: *E. coli*, **Ec[a]**: *E. coli* O157:H7, **St**: *Salmonella typhimurium*, Gram positives; **Bc**: *Bacillus cereus*, **Bs**: *Bacillus subtilis*, **Sa**: *Staphylococcus aureus*, **Lm**: *Listeria monocytogenes*, **Se**: *Staphylococcus epidermidis*

Anticandidal activity tests were performed against *Candida zeylanoides*, *C. utilis*, and two different strains of *Candida albicans*, *C. krusei*, *C. parapsilosis*, *C. glabrata* and *C. tropicalis*. *C. krusei* (ATCC 6258) and *C. parapsilosis* (ATCC® 22019) were included in the test panel as quality control strains recommended by CLSI M27-A2 protocol. MIC results obtained anticandidal tests were read at both 24 and 48 h and represented in different tables (Table 5-10). According to test results, monoterpene hydrocarbons, aldehydes, ketones and esters showed weak to moderate anticandidal effects between the concentrations of 8 to 0.06 mg/mL. Remarkably (1*S*)-(-)-*α*-Pinene, (-)-camphene, *p*-cymene, (-)-limonene, citral, myrtenal and (1*S*)-(-)-camphor were the most active compounds among the monoterpene hydrocarbons, aldehydes and ketones. Esters generally showed weak effects against *Candida* panel. (+)-isomenthol, (*S*)-(−)-*α*-terpineol, lavandulol, cumin (or cuminic) alcohol, carvacrol and thymol (1 to 0.06 mg/mL) were the most active phenolic and monoterpene alcohols. Significantly, after 48h incubation period isomenthol, carvacrol, thymol and thymoquinone were effective against the tested pathogenic yeasts (Table 7 and 8).

Apart from monoterpenes, estragol, *trans*-anethol and thymoquinone were determined as strong anticandidal constituents. For both 24 and 48 incubation periods, thymoquinone was determined as the most active anticandidal compound (0.01-0.03 mg/mL, MIC) among the tested

constituents. Minus isomers (-) of limonene, *α*-pinene, camphene, *β*-citronellol, *α*-terpineol, menthol, menthone, pulegone, camphor and carvone clearly exhibited stronger anticandidal effects than their plus isomers. On the contrary, plus isomers (+) of the *β*-pinene, terpinen-4-ol and citronellal were found as more active isomers against the pathogens studied. In a previous study, (+)-*β*-pinene was found more toxic to *Candida albicans* cells than its minus isomer similar to our results [47]. Generally, tested compounds demonstrated better inhibitory effects on *Candida* strains than the bacteria strains.

Table 4. Antibacterial Activity of Aldehydes, Ketones, Esters and others (MIC, mg/mL)

	Gram (-)						Gram (+)					
Compounds	**Pa**	**Ea**	**Pv**	**Sm**	**Ec**	**Ec[a]**	**St**	**Bc**	**Bs**	**Sa**	**Lm**	**Se**
Aldehydes and Ketones												
(*R*)-(+)-Citronellal	4	16	4	4	16	8	4	4	0.25	4	2	4
(*S*)-(-)-Citronellal	4	16	4	4	16	8	4	4	1	4	2	2
(1*R*)-(-)-Myrtenal	4	16	1	4	16	8	8	1	0.5	4	1	2
Citral	4	4	1	4	4	8	8	1	0.5	2	1	2
Cuminaldehyde	4	4	4	4	16	8	4	4	2	4	2	2
(-)-Menthone	4	8	4	4	8	4	16	4	4	2	8	8
(+)-Menthone	4	16	4	4	8	4	16	8	4	4	8	8
(*R*)-(+)-Pulegone	4	16	4	4	8	4	8	2	4	2	4	4
(*S*)-(-)-Pulegone	4	16	4	4	8	4	8	4	4	2	4	4
(*R*)-(-)-Carvone	2	8	4	2	8	4	8	4	4	2	4	8
(*S*)-(+)-Carvone	4	16	16	4	8	4	8	8	4	4	4	8
(1*S*)-(-)-Verbenone	2	2	2	1	1	1	1	0.5	4	1	1	2
(±) Camphor	2	2	2	2	2	2	8	2	2	8	2	4
(1*R*)-(+)-Camphor	2	2	2	2	2	2	8	4	2	8	2	4
(1*S*)-(-)-Camphor	2	2	2	1	2	2	8	4	2	8	2	4
Esters												
Geranyl acetate	16	16	4	4	16	4	4	4	4	4	8	8
l-Menthyl acetate	16	16	4	4	8	4	4	4	4	4	4	8
(-)-Bornyl acetate	2	16	4	4	8	4	4	8	8	4	8	8
(+)-Bornyl acetate	16	16	4	4	8	4	8	4	8	4	4	8
Isobornyl acetate	8	16	4	8	8	4	4	8	4	4	8	8
Linalyl acetate	4	16	4	4	8	4	16	4	4	4	8	8
Phenylpropanoids and other oxygenated monoterpenes												
Estragol	2	2	4	2	2	4	4	4	2	4	2	2
trans -Anethol	4	4	2	4	2	4	4	4	8	4	4	2
Eugenol	4	4	2	2	2	2	4	1	0.5	4	2	4
1,8-cineole	2	1	1	2	2	2	1	1	2	1	8	2
Thymoquinone	1	1	0.03	1	0.5	1	1	0.03	0.06	0.03	0.06	0.03
St-1	0.128	0.032	0.001	0.016	0.002	0.001	0.001	0.002	0.001	0.001	0.001	0.001
St-2	0.064	0.002	0.004	0.004	0.001	0.001	0.001	0.004	0.001	0.008	0.002	0.001

[1]: O157:H7; St-1: Ampicillin, St-2: Chloramphenicol

Antifungal action mechanisms of essential oils and their constituents are generally explained by membrane damage or disruption of its integrity, increasing permeability, inhibition of ergosterol synthesis or binding to ergosterol on the membrane and ROS production by acting on mitochondria [26, 48-50]. Our previous electron microscopy study has revealed the extensive cell wall and cytoplasmic membrane damage after exposure to thymoquinone that is major component of the black cumin seed essential oil [51]. Most of the antifungal activity in those essential oil is attributed to oxygenated monoterpenes especially alcohols and phenols, some monoterpene hydrocarbons and phenylpropanoids. So many studies reported that the interactions between these constituents in essential oils resulting synergistic, antagonistic or additive effects [25].

The mechanism of action of monoterpenes is not fully understood but it is generally accepted that the microorganisms are inhibited by essential oils due to their effects on membrane integrity and functions

[18]. Hydrophobic constituents of the essential oils restrict the cell diffusion, respiratory, biofilm formation and quorum sensing especially in several concentrations (5% to 0.0025% v/v). It has been reported that a 0.5% concentration of tea-tree oil completely inhibited the respiration of *E. coli*.

Table 5. Anticandidal Activity Results of Monoterpene Hydrocarbons (**24h**, MIC, mg/mL)

Compounds	**Ca^a**	**Ca^b**	**Cu**	**Ck^a**	**Ck^b**	**Cz**	**Cp^a**	**Cp^b**	**Cg^a**	**Cg^b**	**Ct^a**	**Ct^b**
p-Cymene	0.25	0.25	0.12	0.12	0.12	0.5	0.5	0.25	0.25	0.25	0.25	0.06
Ocimene	4	4	4	4	4	2	4	4	4	8	8	1
Myrcene	2	2	0.5	1	1	0.5	1	4	1	2	4	0.25
(*R*)-(+)-Limonene	2	1	0.25	0.25	0.25	0.5	2	1	0.5	1	1	0.5
(*S*)-(-)-Limonene	1	1	0.12	0.12	0.25	0.5	0.5	0.5	0.25	0.5	0.5	0.12
Terpinolene	4	4	1	4	2	4	2	4	4	4	4	1
γ-Terpinene	4	4	2	4	4	4	8	8	8	8	8	1
(+)-3-Carene	2	2	0.12	0.5	0.5	1	0.5	1	1	1	2	0.25
Sabinene	4	8	2	2	4	4	4	8	4	4	8	0.25
(1*R*)-(+)-*α*-Pinene	0.5	1	0.12	0.25	0.5	0.5	0.5	0.5	0.5	1	1	0.12
(1*S*)-(-)-*α*-Pinene	0.5	1	0.12	0.25	0.5	0.5	0.5	0.5	0.25	0.5	0.5	0.12
(+)-*β*-Pinene	1	1	0.25	0.25	0.25	0.5	0.5	1	0.5	1	0.5	0.06
(-)-*β*-Pinene	2	4	0.5	1	2	1	2	2	2	1	4	0.12
(+)-Camphene	1	2	1	1	2	1	1	2	2	2	4	0.5
(-)-Camphene	0.5	1	0.5	0.5	0.12	0.12	0.5	0.5	0.25	0.5	2	0.12
α-Pinene	1	4	0.12	0.5	0.5	1	2	1	1	1	4	0.06
α-Phellandrene	4	1	2	2	2	2	4	4	2	4	4	0.25
St-3	5×10^{-4}	1×10^{-3}	25×10^{-5}	$0{,}5\times10^{-3}$	1×10^{-3}	5×10^{-4}	1×10^{-3}	5×10^{-4}	1×10^{-3}	1×10^{-3}	1×10^{-3}	1×10^{-3}
St-4	6×10^{-5}	6×10^{-5}	6×10^{-5}	25×10^{-5}	25×10^{-5}	6×10^{-5}	6×10^{-5}	6×10^{-5}	6×10^{-5}	12×10^{-5}	6×10^{-5}	6×10^{-5}

St-3: Amphotericin-B, St-4: Ketoconazole, **Ca^a** :*Candida albicans* ATCC 10231, **Ca^b**: *Candida albicans* ATCC 24433, **Cu**: *Candida utilis* NRRL Y-900, **Ck^a**: *Candida krusei* NRRL Y-7179, **Ck^b**: *Candida krusei* ATCC 6258, **Cz**: *Candida zeylanoides* NRRL Y-1774, **Cg^a**: *Candida glabrata* ATCC 2001, **Cg^b**: *Candida glabrata* ATCC 66032, **Ct^a**: *Candida tropicalis* ATCC 1369, **Ct^b**: *Candida tropicalis* ATCC 750, **Cp^a**: *Candida parapsilosis*; NRRL Y- 12696, **Cp^b**: *Candida parapsilosis* ATCC 22019

Table 6. Anticandidal Activity Results of Monoterpene Hydrocarbons (48h, MIC, mg/mL)

Compounds	**Ca^a**	**Ca^b**	**Cu**	**Ck^a**	**Ck^b**	**Cz**	**Cp^a**	**Cp^b**	**Cg^a**	**Cg^b**	**Ct^a**	**Ct^b**
p-Cymene	0.5	0.25	0.25	0.12	0.12	2	1	0.12	0.25	0.5	0.25	0.06
Ocimene	8	8	8	4	8	8	8	8	8	8	8	2
Myrcene	4	2	2	0.5	2	2	2	4	4	4	4	0.5
(*R*)-(+)-Limonene	2	2	0.5	0.25	0.25	1	2	1	1	1	1	0.5
(*S*)-(-)-Limonene	2	1	0.25	0.25	0.25	1	1	1	0.5	0.5	1	0.12
Terpinolene	8	8	2	8	8	8	4	8	4	4	8	2
γ-Terpinene	8	8	8	8	8	8	8	8	8	8	8	2
(+)-3-Carene	2	4	0.25	0.5	1	2	0.5	1	1	1	2	0.25
Sabinene	8	8	4	4	8	4	8	8	8	8	8	0.5
(1*R*)-(+)-*α*-Pinene	0.5	1	0.25	0.5	0.5	1	1	1	1	1	2	0.25
(1*S*)-(-)-*α*-Pinene	0.5	1	0.25	0.5	0.5	1	0.5	1	0.5	1	1	0.25
(+)-*β*-Pinene	1	1	0.5	0.5	0.25	1	1	1	1	1	1	0.12
(-)-*β*-Pinene	2	4	1	2	2	1	2	4	2	2	8	0.5
(+)-Camphene	2	2	2	2	4	2	4	4	4	2	4	0.5
(-)-Camphene	1	2	0.5	0.5	0.25	0.25	1	1	0.5	1	2	0.5
α-Pinene	2	4	0.25	0.5	1	2	4	1	2	2	8	0.12
α-Phellandrene	8	2	2	2	2	2	4	8	4	8	8	0.5
St-3	5×10^{-4}	2×10^{-3}	5×10^{-4}	1×10^{-3}	2×10^{-3}	1×10^{-3}	1×10^{-3}	1×10^{-3}	2×10^{-3}	2×10^{-3}	2×10^{-3}	2×10^{-3}
St-4	6×10^{-5}	12×10^{-5}	25×10^{-5}	5×10^{-4}	5×10^{-4}	6×10^{-5}	12×10^{-5}	6×10^{-5}	6×10^{-5}	5×10^{-4}	6×10^{-5}	6×10^{-5}

St-3: Amphotericin-B, St-4: Ketoconazole, **Ca^a** :*Candida albicans* ATCC 10231, **Ca^b**: *Candida albicans* ATCC 24433, **Cu**: *Candida utilis* NRRL Y-900, **Ck^a**: *Candida krusei* NRRL Y-7179, **Ck^b**: *Candida krusei* ATCC 6258, **Cz**: *Candida zeylanoides* NRRL Y-1774, **Cg^a**: *Candida glabrata* ATCC 2001, **Cg^b**: *Candida glabrata* ATCC 66032, **Ct^a**: *Candida tropicalis* ATCC 1369, **Ct^b**: *Candida tropicalis* ATCC 750, **Cp^a**: *Candida parapsilosis*; NRRL Y- 12696, **Cp^b**: *Candida parapsilosis* ATCC 22019

Generally, essential oils act in a short time period and can be lethal for microorganisms in higher doses while display nonlethal effects and need more time to acting in lower doses. Some of the constituents such as carvacrol, thymol, *p*-cymene, 1,8-cineole, terpinene-4-ol and α-terpinene were reported as membrane fluidity increasing agents. These constituents act in minutes (15 to 30 min) and increase the fluidity, which is cause leakage of potassium and sodium ions flow through the membrane [24].

Furthermore essential oils affect the solubility and intake of trace ions around the cell. As a result of this, availability of the trace elements, such as iron, are reduced and inhibited the growth of the cells.

This study reports effectiveness of the essential oil components as a pure molecule against several clinical and foodborne pathogens.

Table 7. Anticandidal Activity Results of the Monoterpene Alcohols (24h, MIC, mg/mL)

Compounds	**Ca^a**	**Ca^b**	**Cu**	**Ck^a**	**Ck^b**	**Cz**	**Cp^a**	**Cp^b**	**Cg^a**	**Cg^b**	**Ct^a**	**Ct^b**
(-)-Linalool	0.5	1	1	2	2	2	1	2	2	2	2	2
Geraniol	0.06	0.5	0.25	1	1	1	0.5	0.5	1	1	1	1
Nerol	0.12	1	0.25	1	1	1	1	1	1	1	1	1
(*S*)-(-)-*β*-Citronellol	0.06	1	1	1	1	0.5	1	1	2	2	1	0.5
(*R*)-(+)-*β*-Citronellol	0.12	1	1	2	1	1	2	1	2	2	2	2
(+)-Isomenthol	0.12	0.12	0.12	0.12	0.12	0.25	0.25	0.12	0.25	1	0.5	0.25
(+)-Neomenthol	0.5	2	2	1	2	2	1	2	2	2	2	2
(-)-Menthol	0.5	0.5	0.25	0.5	0.5	0.5	0.5	1	1	1	1	1
(+)-Menthol	0.5	1	0.5	0.5	1	1	0.5	1	1	1	1	1
(+)-Isopulegol	0.5	1	0.25	0.5	0.5	1	0.5	0.5	1	1	1	1
(-)-Isopulegol	0.5	1	0.25	0.5	0.5	1	0.5	0.5	1	1	1	1
(*R*)-(+)-α-Terpineol	0.25	1	0.25	0.5	1	0.5	0.5	0.5	0.5	1	2	0.25
(*S*)-(−)-α-Terpineol	0.5	1	0.25	0.5	0.5	1	0.25	0.5	0.12	1	1	0.12
(-)-Terpinen-4-ol	0.5	2	0.5	1	1	1	1	1	1	2	2	1
(+)-Terpinen-4-ol	0.5	1	0.25	1	1	0.5	0.5	1	0.5	0.5	1	0.12
(-)-Carveol	0.12	0.5	0.25	0.5	0.5	0.5	0.5	0.5	0.5	1	0.5	1
Cumic alcohol	0.06	0.5	0.25	0.5	0.5	0.25	0.25	0.25	0.5	1	0.5	0.5
(1*R*)-(-)-Myrtenol	0.25	0.25	0.5	1	1	0.5	0.5	1	1	1	1	0.5
(*S*)-*cis*-Verbenol	1	0.25	0.5	0.5	1	0.5	0.5	1	0.5	1	1	1
(+)-Borneol	1	0.5	0.5	0.5	0.5	1	0.5	1	0.5	1	1	1
(-)-Borneol	1	0.5	0.5	0.5	0.5	1	1	1	0.5	1	1	1
(±)-Isoborneol	1	1	0.5	0.5	0.5	1	1	1	1	1	1	2
Lavandulol	0.5	1	0.25	0.5	0.5	0.12	0.25	0.5	0.5	1	0.5	0.25
Carvacrol	0.12	0.25	0.06	0.12	0.12	0.12	0.06	0.06	0.12	0.25	0.12	0.25
Thymol	0.06	0.25	0.12	0.12	0.25	0.12	0.25	0.12	0.25	0.25	0.25	0.5
St-3	5×10^{-4}	1×10^{-3}	25×10^{-5}	0.5×10^{-3}	1×10^{-3}	5×10^{-4}	1×10^{-3}	5×10^{-4}	1×10^{-3}	1×10^{-3}	1×10^{-3}	1×10^{-3}
St-4	6×10^{-5}	6×10^{-5}	6×10^{-5}	25×10^{-5}	25×10^{-5}	6×10^{-5}	6×10^{-5}	6×10^{-5}	6×10^{-5}	12×10^{-5}	6×10^{-5}	6×10^{-5}

St-3: Amphotericin-B, St-4: Ketoconazole, **Ca^a** :*Candida albicans* ATCC 10231, **Ca^b**: *Candida albicans* ATCC 24433, **Cu**: *Candida utilis* NRRL Y-900, **Ck^a**: *Candida krusei* NRRL Y-7179, **Ck^b**: *Candida krusei* ATCC 6258, **Cz**: *Candida zeylanoides* NRRL Y-1774, **Cg^a**: *Candida glabrata* ATCC 2001, **Cg^b**: *Candida glabrata* ATCC 66032, **Ct^a**: *Candida tropicalis* ATCC 1369, **Ct^b**: *Candida tropicalis* ATCC 750, **Cp^a**: *Candida parapsilosis*; NRRL Y- 12696, **Cp^b**: *Candida parapsilosis* ATCC 22019

Table 8. Anticandidal Activity Results of the Monoterpene Alcohols (48h, MIC, mg/mL)

Compounds	**Ca^a**	**Ca^b**	**Cu**	**Ck^a**	**Ck^b**	**Cz**	**Cp^a**	**Cp^b**	**Cg^a**	**Cg^b**	**Ct^a**	**Ct^b**
(-)-Linalool	0.5	1	1	2	4	2	2	4	2	4	4	2
Geraniol	0.06	0.5	0.5	2	2	1	1	1	2	2	2	1
Nerol	0.25	1	0.5	2	2	1	2	2	2	2	1	1
(*S*)-(-)-*β*-Citronellol	0.06	1	2	2	2	1	1	2	2	2	2	0.5
(*R*)-(+)-*β*-Citronellol	0.12	1	2	2	2	1	4	2	4	2	4	2
(+)-Isomenthol	0.12	0.12	0.25	0.25	0.25	0.5	0.5	0.25	0.5	0.5	0.5	0.25
(+)-Neomenthol	0.5	2	2	2	4	2	1	2	2	2	4	2
(-)-Menthol	0.5	1	1	1	1	1	1	1	2	2	1	1
(+)-Menthol	0.5	1	1	1	1	2	1	1	2	2	1	1
(+)-Isopulegol	1	2	0.5	1	1	1	1	1	2	2	1	1
(-)-Isopulegol	2	2	1	1	1	1	1	1	2	1	2	1
(*R*)-(+)-*α*-Terpineol	0.25	1	0.25	0.5	2	0.5	1	1	1	2	2	0.25
(*S*)-(−)-*α*-Terpineol	0.5	1	0.5	1	1	0.25	0.5	1	1	2	2	0.12
(-)-Terpinen-4-ol	0.5	2	1	2	2	1	2	1	2	2	2	1
(+)-Terpinen-4-ol	0.5	1	0.5	2	2	1	1	1	2	2	2	0.5
(-)-Carveol	0.25	0.5	0.5	1	1	0.5	0.5	1	1	1	1	1
Cumin alcohol	0.06	1	0.5	0.5	1	0.5	0.5	0.5	1	1	1	0.5
(1*R*)-(-)-Myrtenol	0.5	0.5	1	1	2	0.5	1	2	1	1	1	0.5
(*S*)-*cis*-Verbenol	1	0.5	0.5	1	1	1	1	1	1	1	1	1
(+)-Borneol	1	0.5	1	1	1	1	1	2	1	1	1	1
(-)-Borneol	1	0.5	1	1	1	1	1	2	1	1	1	1
(±)-Isoborneol	2	2	1	1	1	1	2	2	2	1	1	2
Lavandulol	0.5	1	1	1	2	0.5	0.5	1	2	2	2	0.25
Carvacrol	0.25	0.25	0.25	0.25	0.25	0.12	0.12	0.12	0.25	0.25	0.25	0.25
Thymol	0.25	0.5	0.25	0.5	0.5	0.25	0.25	0.25	0.5	0.25	0.5	0.5
St-3	5×10^{-4}	2×10^{-3}	5×10^{-4}	1×10^{-3}	2×10^{-3}	1×10^{-3}	1×10^{-3}	1×10^{-3}	2×10^{-3}	2×10^{-3}	2×10^{-3}	2×10^{-3}
St-4	6×10^{-5}	12×10^{-5}	25×10^{-5}	5×10^{-4}	5×10^{-4}	6×10^{-5}	12×10^{-5}	6×10^{-5}	6×10^{-5}	5×10^{-4}	6×10^{-5}	6×10^{-5}

St-3: Amphotericin-B, St-4: Ketoconazole, **Ca^a** :*Candida albicans* ATCC 10231, **Ca^b**: *Candida albicans* ATCC 24433, **Cu**: *Candida utilis* NRRL Y-900, **Ck^a**: *Candida krusei* NRRL Y-7179, **Ck^b**: *Candida krusei* ATCC 6258, **Cz**: *Candida zeylanoides* NRRL Y-1774, **Cg^a**: *Candida glabrata* ATCC 2001, **Cg^b**: *Candida glabrata* ATCC 66032, **Ct^a**: *Candida tropicalis* ATCC 1369, **Ct^b**: *Candida tropicalis* ATCC 750, **Cp^a**: *Candida parapsilosis*; NRRL Y- 12696, **Cp^b**: *Candida parapsilosis* ATCC 22019

When safety issues are considered, pure compounds may be gained more importance using practically in pharmaceutical, fragrance and food systems. Due to the toxic effects of some constituents in essential oils, some of them are not considered as safe. Hundreds of publishing studies have shown that essential oils and their single components are more or less effective against several pathogenic bacteria. Furthermore, in many studies reported that the synergism has been observed between a pure essential oil constituent and its precursor and between the well-known antibiotics and pure compounds. In addition to that, efficacy of the essential oils and their constituents may improve by modifying the physical conditions (pH, temperature and oxygen levels). Consequently, by the further studies on the parameters like synergism, modifying conditions etc may provide new findings on their efficacy and practical use.

Table 9. Anticandidal activity of aldehydes, ketones, esters and others (24h, MIC, mg/mL)

Compounds	**Ca^a**	**Ca^b**	**Cu**	**Ck^a**	**Ck^b**	**Cz**	**Cp^a**	**Cp^b**	**Cg^a**	**Cg^b**	**Ct^a**	**Ct^b**
Aldehydes and Ketones												
(*R*)-(+)-Citronellal	1	1	2	0.5	1	1	1	1	2	2	2	1
(*S*)-(-)-Citronellal	0.5	2	2	0.5	1	2	2	1	1	1	2	1
(1*R*)-(-)-Myrtenal	0.5	1	0.25	0.5	1	2	1	0.5	1	1	2	1
Citral	0.25	1	0.25	0.25	0.25	1	1	0.25	0.5	0.5	1	0.5
Cuminaldehyde	1	1	2	0.5	1	2	2	1	1	2	4	1
(-)-Menthone	4	2	2	1	2	4	2	2	1	1	2	1
(+)-Menthone	4	4	0.5	2	1	8	4	2	2	2	4	0.5
(*R*)-(+)-Pulegone	1	2	1	1	1	2	1	1	2	2	4	0.5
(*S*)-(-)-Pulegone	1	1	0.25	0.5	0.5	2	0.5	1	2	2	2	0.25
(*R*)-(-)-Carvone	2	4	1	1	1	4	2	1	2	2	2	1
(*S*)-(+)-Carvone	2	4	1	1	1	2	2	1	2	2	2	1
(1*S*)-(-)-Verbenone	1	1	1	1	1	2	2	1	1	2	1	1
(±) Camphor	0.5	0.5	1	1	2	1	1	0.5	2	0.5	1	0.5
(1*R*)-(+)-Camphor	0.5	0.5	1	1	1	1	1	1	1	1	1	0.5
(1*S*)-(-)-Camphor	1	1	0.5	0.5	0.5	4	2	0.5	0.25	1	1	0.5
Esters												
Geranyl acetate	0.5	4	2	0.5	0.5	4	2	1	1	1	8	0.25
l-Menthyl acetate	4	4	2	4	1	4	4	2	1	2	4	4
(-)-Bornyl acetate	4	2	2	2	1	8	4	4	2	2	4	4
(+)-Bornyl acetate	4	4	2	2	4	4	4	2	4	4	4	4
Isobornyl acetate	1	2	1	1	4	1	1	2	2	1	4	4
Linalyl acetate	2	8	8	1	1	4	2	1	2	2	4	0.5
Phenylpropanoids and other oxygenated monoterpenes												
Estragol	0.12	0.12	0.06	0.06	0.06	0.12	0.25	0.06	0.06	0.12	0.12	0.06
trans-Anethol	0.12	0.25	0.5	0.25	0.25	0.5	0.25	0.5	0.25	0.5	0.5	0.5
Eugenol	2	2	1	1	2	2	2	2	4	4	4	2
1,8-cineole	2	4	2	2	4	4	2	2	2	2	2	1
Thymoquinone	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.06	0.06	0.03	0.03
St-3	5×10^{-4}	1×10^{-3}	25×10^{-5}	0.5×10^{-3}	1×10^{-3}	5×10^{-4}	1×10^{-3}	5×10^{-4}	1×10^{-3}	1×10^{-3}	1×10^{-3}	1×10^{-3}
St-4	6×10^{-5}	6×10^{-5}	6×10^{-5}	25×10^{-5}	25×10^{-5}	6×10^{-5}	6×10^{-5}	6×10^{-5}	6×10^{-5}	12×10^{-5}	6×10^{-5}	6×10^{-5}

St-3: Amphotericin-B, St-4: Ketoconazole, **Ca^a** :*Candida albicans* ATCC 10231, **Ca^b**: *Candida albicans* ATCC 24433, **Cu**: *Candida utilis* NRRL Y-900, **Ck^a**: *Candida krusei* NRRL Y-7179, **Ck^b**: *Candida krusei* ATCC 6258, **Cz**: *Candida zeylanoides* NRRL Y-1774, **Cg^a**: *Candida glabrata* ATCC 2001, **Cg^b**: *Candida glabrata* ATCC 66032, **Ct^a**: *Candida tropicalis* ATCC 1369, **Ct^b**: *Candida tropicalis* ATCC 750, **Cp^a**: *Candida parapsilosis*; NRRL Y- 12696, **Cp^b**: *Candida parapsilosis* ATCC 22019

Table 10. Anticandidal Activity of aldehydes, ketones, esters and phenyl propanoids (48h, MIC, mg/mL)

Compounds	**Ca^a**	**Ca^b**	**Cu**	**Ck^a**	**Ck^b**	**Cz**	**Cp^a**	**Cp^b**	**Cg^a**	**Cg^b**	**Ct^a**	**Ct^b**
Aldehydes and Ketones												
(*R*)-(+)-Citronellal	2	2	4	1	2	1	4	2	4	4	4	2
(*S*)-(-)-Citronellal	2	8	4	2	2	2	2	2	2	2	8	4
(1*R*)-(-)-Myrtenal	2	2	0.5	2	4	2	1	1	2	2	4	4
Citral	2	4	0.5	1	0.5	1	1	0.5	2	1	2	1
Cuminaldehyde	4	2	4	2	4	2	2	4	4	4	8	4
(-)-Menthone	8	4	8	4	4	8	4	4	4	4	4	2
(+)-Menthone	8	8	2	8	4	8	8	4	4	8	8	2
(*R*)-(+)-Pulegone	4	4	2	4	4	2	4	2	4	2	8	1
(*S*)-(-)-Pulegone	4	2	1	1	1	4	1	2	4	4	4	0.5
(*R*)-(-)-Carvone	4	8	2	2	2	2	4	2	4	4	4	4
(*S*)-(+)-Carvone	4	8	2	4	4	2	4	2	4	8	4	4
(1*S*)-(-)-Verbenone	2	2	2	2	4	4	4	2	2	4	2	2
(±) Camphor[2]	1	2	2	2	2	2	2	2	2	2	2	2
(1*R*)-(+)-Camphor	1	1	2	2	2	2	2	2	2	2	1	1

(1*S*)-(-)-Camphor	2	2	2	2	2	4	2	1	1	4	1	1
Esters												
Geranyl acetate	1	8	4	1	2	4	2	4	4	8	8	1
l-Menthyl acetate	8	8	8	8	4	4	4	4	4	8	4	8
(-)-Bornyl acetate	8	4	4	4	8	8	4	4	8	4	8	4
(+)-Bornyl acetate	8	8	2	4	8	4	4	2	8	8	4	4
Isobornyl acetate	8	8	4	8	4	1	1	8	8	8	8	8
Linalyl acetate	8	8	8	2	4	4	4	2	8	8	8	1
Phenylpropanoids and other oxygenated monoterpenes												
Estragol	0.25	0.5	0.12	0.12	0.06	0.12	0.25	0.12	0.12	0.25	0.25	0.06
trans -Anethol	0.5	1	1	1	1	1	0.5	1	0.5	1	2	2
Eugenol	4	4	2	2	4	4	4	4	4	4	4	4
Eucalyptol	4	4	4	4	4	8	8	4	4	4	4	2
Thymoquinone	0.06	0.03	0.03	0.03	0.03	0.03	0.03	0.06	0.06	0.06	0.03	0.03
St-3	5×10^{-4}	2×10^{-3}	5×10^{-4}	1×10^{-3}	2×10^{-3}	1×10^{-3}	1×10^{-3}	1×10^{-3}	2×10^{-3}	2×10^{-3}	2×10^{-3}	2×10^{-3}
St-4	6×10^{-5}	12×10^{-5}	25×10^{-5}	5×10^{-4}	5×10^{-4}	6×10^{-5}	12×10^{-5}	6×10^{-5}	6×10^{-5}	5×10^{-4}	6×10^{-5}	6×10^{-5}

St-3: Amphotericin-B, St-4: Ketoconazole, **Ca**[a] :*Candida albicans* ATCC 10231, **Ca**[b]: *Candida albicans* ATCC 24433, **Cu**: *Candida utilis* NRRL Y-900, **Ck**[a]: *Candida krusei* NRRL Y-7179, **Ck**[b]: *Candida krusei* ATCC 6258, **Cz**: *Candida zeylanoides* NRRL Y-1774, **Cg**[a]: *Candida glabrata* ATCC 2001, **Cg**[b]: *Candida glabrata* ATCC 66032, **Ct**[a]: *Candida tropicalis* ATCC 1369, **Ct**[b]: *Candida tropicalis* ATCC 750, **Cp**[a]: *Candida parapsilosis*; NRRL Y- 12696, **Cp**[b]: *Candida parapsilosis* ATCC 22019

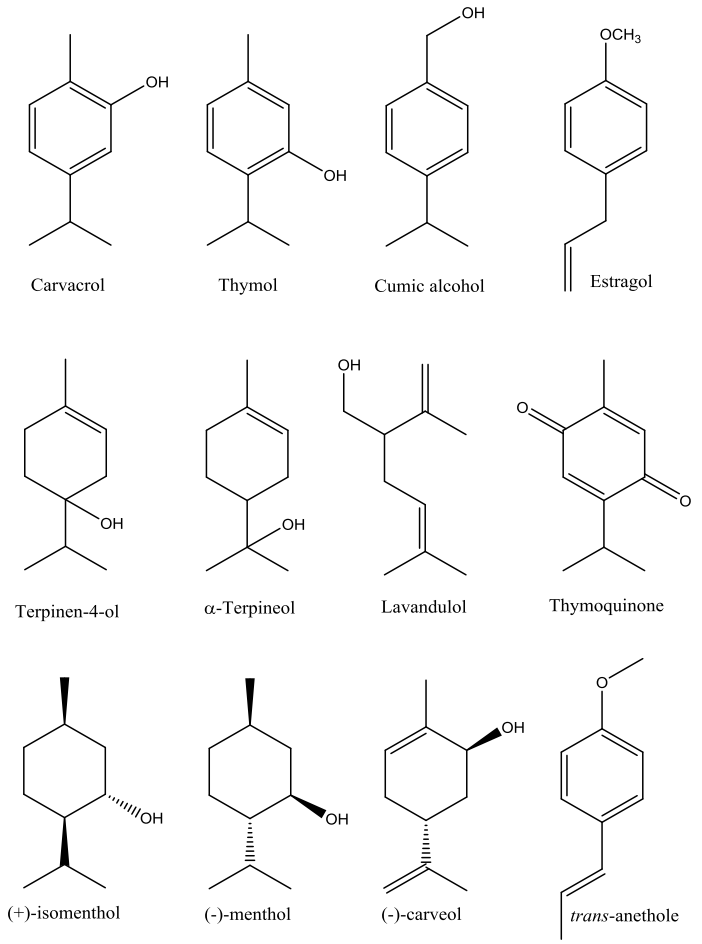

Figure 1. Most active compounds against tested bacteria and *Candida* strains.

4. Conclusion

According to test results, as expected terpene alcohols, phenols and aldehydes appeared to be the most active components while terpene hydrocarbons and esters appeared to be the least active compounds tested (Figure 1). Observed activity of the terpene alcohols may be attributed to high solubility ratio of these compounds in both aqueous media and bio membranes due to the alcohol moiety. Some differentiations were observed on the antimicrobial properties of stereoisomers. With this study, it was revealed that the minus isomers of the monoterpenoids generally demonstrated stronger antibacterial effects.

Gram negative strains especially *Pseudomonas aeruginosa* and *Enterobacter aerogenes* demonstrated less susceptibility against the essential oil components. Generally, tested compounds demonstrated better inhibitory effects on *Candida* strains than the bacteria.

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