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Research Article

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Chemical Composition, Antifungal and Antioxidant Activity of Some Spice Essential Oils

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Abstract: This study was carried out to determine the chemical composition of some spice essential oils, to test the radical scavenging and the anti-fungal activities of Cinnamon, Anise, Cumin and Caraway EOs. The essential oil compositions were determined by GC-MS and showed variation in its constituent for each type of oil. Major identified components were Cinnamaldehyde 81.79 % for Cinnamon EO (Ci-EO), Anethole 90.9% for Anise EO (An-EO), (gamma-Terpinene 23.84%, beta.-Pinene 21.84%, Benzaldehyde, 4-(1-methylethyl) 20.00%, Carenal 17.50% and Benzene, 1-methyl-3-(1-methylethyl) 14.10%) for Cumin EO (Cu-EO) and (Carvone 52.27% and D-Limonene 47.13 %) for Caraway EO (Ca-EO). Antioxidant effectiveness was examined by the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method using different concentrations of EOs (2.5, 5.0, 10.0 , 20.0 , 40.0 , mg/ml) at different times. All essential oils have shown antioxidant activity. Based on their antioxidant capacity, four spice essential oils sorted in descending order: Cumin 87.4% > Anise 72.4% > Caraway 59.5 > Cinnamon18.2 % at high concentration 40.0, mg/ml at 120 min. These essential oils were also found to possess remarkable antifungal activity against tested pathogens like yeasts (*Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 22019) and fungi (*Aspergillus niger, Aspergillus flavus* and *Aspergillus fumigatus)* by using disc diffusion technique. Cu- EO showed 100% inhibition against *A. niger*, *C. krusei* and *C. parapsilosis*. For other tested fungi, the essential oils showed good to moderate inhibitory effects. Positive and important associations between mycelial growth inhibition of both pathogens and the antioxidant activity of the checked oils. These results showed tested essential oils could be used as a source of antioxidant and antifungal compounds which may find applications in pharmaceutical and food industries.

Keywords: Spices Essential Oils, Chemical Composition, Antioxidant Activity, Antifungal Activity.

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1. INTRODUCTION

Essential oils are aromatic volatile oily hydrophobic concentrate fluid derived from product of plants such as seeds, leaves, twigs, buds, flowers, bark, wood, roots, fruits and whole crop, that may contain over 100 different components of 20 to 60 volatile compounds in a form of complex mixtures.[1,2] Since ancient times, antimicrobial and antioxidant properties of essential oils from aromatic and medicinal plants are recognized, and the spices were designed for various purposes, such as flavouring, holding insects away and in perfumery.[3] Fungi are one of the most critical indoor air pollutants in residential and industrial environment, which can be a significant factor developing infectious and harmful diseases in plants and animals, including humans.[4, 5] Presence of Penicillium fungi in air environment of Food Industries, may expose the ultimate consumers of these products to possible risk. The fungus is usually linked with contaminated food, showing the economic losses arising from mycotoxin production which can harm for human health.[6] In order to prove the impact of essential oil and its major compounds on pathogenic fungi, numerous studies have been conducted. Kurita *et al.,* tested 40 plant compounds against seven fungal species and found highly effective plant metabolites against fungal pathogens.[7]*Cuminum cyminum* (Cumin), the world's second most common spice after black pepper, is grown primarily in India, China, Arabia as well as Mediterranean countries .[8]Cumin's medicinal uses include, use against indigestion, flatulence, and diarrhea as a stimulant and as carminative, astringent.[9] Studies of Cu-EO's antimicrobial efficacy against a variety of food pathogens are available, but there is a lack of EO's practical efficiency in the food system as a preservative.[8, 10-11] *Cinnamomum verum* commonly referred as cinnamon, was known from ancient times as other volatile oils in medicine and had a reputation for being a cold cure. Ci-EO also has antimicrobial properties that can help preserve other foods.[12] While oil from all over the world has demonstrated a great variety of chemical constituents, this mainly consists of trans-cinnamaldehyde (47-71%) as the major component.[13]*C. verum* EO has good antibacterial and antimicrobial activity alone [14] or in combination with other essential oils in various studies.[15]EOs may have antioxidant properties and their use can affect the functions of immune cells. In the food industry, antioxidants are used to increase food shelf-life. Antioxidants can also stop the reaction in the human body of free radicals with biomolecules; minimizing cell injury and death, chronic and cardiovascular diseases, and so on.[16]These may also be used in the food industry to replace artificial food additives that are antioxidant. Natural antioxidants have increased interest among consumers and the communities for researchers, especially in spices, since epidemiological studies have shown that regular use of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer.[17,18]The aim of the present study is to determine (i)chemical composition (ii)antifungal activity(iii) antioxidant activity of essential oils of *Cinnamomum verum* (Cinnamon), *Pimpinella anisum* (Anise), *Cuminum cyminum* (Cumin) and *Carum carvi* (Caraway).

2. MATERIALS AND METHODS

2.1 Essential oil sample

Essential oil samples of *Cinnamomum verum*, *Cuminum cyminum, Pimpinella anisum and Carum carvi* were purchased from local market in Jeddah, Saudi Arabia.

2.2 Tested fungal strains

Candida krusei ATCC (6258), *Candida parapsilosis* ATCC (22019), *Aspergillus niger, Aspergillus flavus and Aspergillus fumigatus.* The fungal strains were obtained from King AbdulAziz University Hospital, Jeddah, Saudi Arabia.

2.3 GC–mass spectrometry analysis

The GC-MS (Agilent Technologies) device was fitted with GC (7890B) and Mass spectrometer detector (5977A). Dilution was made for samples using hexane (1:19, v/v). GC was fitted with HP-5 MS column (internal diameter 30 mx 0.25 mm and film thickness 0.25 µm). Analyzes was performed by using carrier gas (Helium) at 1.0 ml / min flow rate at a split ratio of 1:10, injected volume was1 µl and the following temperature schedule (40 / 1; 4 / 1 to 150/ 6; 4°C / 1 to 210/ 5) °C/ min. Injector and detector are placed respectively at (280&220) °C., respectively. Through electron ionization (EI) at 70 eV, mass spectra were obtained by using spectral range of m / z 40-550 and solvent delay of 3 min. Specific constituent classification was calculated through making comparison between spectrum fragmentation pattern and data stored in the Wiley and NIST Mass Spectral Library.

2.4 Free radical scavenging activity

Antioxidant activity of the four spices EOs was determined using DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging based on the method of Hae-Ryong.[20]Ascorbic acid was used as references or positive controls. Oils antioxidant activity was measured using the stable DPPH method as updated by Hae-Ryong*et al.,* [19]in terms of hydrogen donation or radical-scavenging efficiency. Reaction mixture containing 2 ml of the (Ci-EO, Cu-EO, An-EO and Ca-EO) at different concentrations "2.5, 5.0, 10.0, 20.0, 40.0, mg/ml" then 2ml of DPPH (0.2mM) was added then shaken strongly after that it was incubated at room temperature in darkness for different periods of time 30, 60, 90 and 120 min. When the DPPH reacted with an antioxidant compound in oil that could donate hydrogen, the UV-visible spectrophotometer decreased the absorbance at 517 nm. The percentage of remaining DPPH was plotted against the concentration of the sample. Further antioxidant activity is suggested by a lower value. Radical scavenging activity was estimated using the following equation as a percentage of inhibition:-

%DPPH "RSA" = [Abs. of Control – Abs. of Sample / Abs. of Control] x 100

Triplicate measurements were conducted, and the results were averaged.

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2.5 Screening of spice essential oils as an antifungal

Testing of essential oils for antifungal activity against tested yeasts (*C. krusei* ATCC 6258, *C. parapsilosis* ATCC 22019) and fungi (*A. niger, A. flavus* and *A. fumigatus)* growth were conducted by disc diffusion method.[20] Mueller Hinton Agar filled 20 ml of previously prepared Petri dishes. Well dried surface was inoculated along the surface by the sterile swab with the conidia suspension. The suspension was spread equally to achieve homogeneous development. For a better absorption of the suspension, inoculated Petri plates are left at room temperature for 15 minutes. Sterile disks (6 mm diameter) were impregnated with 10 µL of essential oil after 15 minutes and left for 30 minutes to avoid unnecessary oil diffusion. The disks are mounted on the Petri plate surface subsequently; inoculated plates were incubated at 37°C/24-48 h for yeast and at 25°C/7 days for fungi. Growth inhibition was measured by calculating the diameter of the inhibition zone (including the diameter of the disk) in mm. The effects of spice essential oils spices on yeast and fungal growth were defined on the basis of calculated inhibition zones and comparison of the obtained values.Triplicate measurements were conducted, and the results were averaged.

3. RESULTS AND DISCUSSION

3.1 Essential oils chemical composition

Table (1) showed the chemical constituents and the relative percentage of the total chromatogram area of Cinnamon Ci-EO, Anise An-EO, Cumin Cu-EO and Caraway Ca-EO. First one was Ci-EO where three compounds were identified, which represents 100 % of the total Ci-EO. The major compounds were cinnamyl aldehyde (81.79%), 2-Propen-1-ol, 3-phenyl-,acetate (14.21%) and Benzaldehyde (4%). These results are in agreement with Wang *et al.,*[21] who reported that the major compound in the cinnamon EO was trans-cinnamyl aldehyde. The next EO was Anise, the results illustrated that An-EO contained nine compounds four of them represent 98.01 % of total An-EO. The major compounds were Anethole (90.9 %), Longifolene (4.2 %), Carvone (1.76 %) and Alpha-pinene, (1.15%). These results are confirmed by others that EO of anise fruits has 80- 95%, or more, *trans*-anethole as the major compound, subsequently chavicol methyl ether (estragole), anisaldehyde and *cis*-anethole.[22,23]After that Cu-EO which contained gamma.-Terpinene (23.84 %), beta.-Pinene (21.84 %), Benzaldehyde, 4-(1-methylethyl)- (20%), 2-Caren-10-al (17.5%), Benzene, 1-methyl-3-(1-methylethyl)- (14.1%), Alpha-pinene (1.15%) they are form 98.43% of Cu-EO. Cuminaldehyde (36–39%) has been identified as main constituent of Cu-EO from China [24], Bulgaria [25] and Tunisia [8] in previous reports. Alpha-pinene (29.2%) has been recorded as major constituent of Iranian Cu-EO.[26]Such variability in the constituent of EO may be due to environmental and geographical features. Caraway was the last EO, where three compounds was found, representing 100% of the total Ca-EO. Carvone (52.27%), Limonene (47.13%) and alpha.-Phellandrene (0.6%) were the main compounds. This finding agree with Khalil *et al.,* who confirmed that Limonene (46.48%) and carvone (50.6%) were the main compounds in the Caraway EO.[27]

Table1. Chemical composition of Cinnamon, Anise, Cumin and Caraway EOs.

Identified compounds	Relative area %			
	Cinnamon	Anise	Cumin	Caraway
Benzaldehyde	04.00	-	-	-
Cinnamaldehyde	81.79	-	-	-
2-Propen-1-ol, 3-phenyl-, acetate	14.21	-	-	-
Linalool	-	1.10	-	-
Carvone	-	1.76	-	52.27
Anethole	-	90.9	-	-
1H-Benzocycloheptene,2,4a,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methylene-, (4aS-cis)-	-	0.36	-	-
Longifolene-(V4)	-	4.20	-	-
Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	-	0.47	-	-
Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	-	0.36	-	-
AlphaLongipinene	-	0.36	-	-
BetaBisabolene	-	0.50	-	-
AlphaThujene	-	-	0.28	-
AlphaPinene	-	-	1.15	-
beta.-Pinene	-	-	21.84	-
Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	-	-	0.58	-
Benzene, 1-methyl-3-(1-methylethyl)-	-	-	14.10	-
gammaTerpinene	-	-	23.84	-
2,6-Dimethyl-3,5,7-octatriene-2-ol, Z,Z-	-	-	0.69	-
Benzaldehyde, 4-(1-methylethyl)-	-	-	20.00	-
2-Caren-10-al	-	-	17.50	-
D-Limonene	-	-	-	47.13
alpha.-Phellandrene	-	-	-	0.60

3.2 Antioxidant activity

Five different working solutions (2.5, 5, 10, 20, 40 mg / mL) were used to assess concentration effect on radical scavenging capacity by DPPH system for EO's of Cinnamon, Anise, Cumin and Caraway. This test was based on the ability to quench DPPH, a stable free radical, and thus decolorize in the presence of antioxidants resulting in reduced absorbance values. Results showed that DPPH scavenging activity increased at different times as shown in Fig. 1 (A, B, C&D) with increased EO concentrations. Evidence shown in Fig.1 (A, B, C&D) showed that the inhibition percentages were increased as concentration and time increased. After 30 min incubation of EO with DPPH Cu-EO showed the highest scavenging activity with increasing EO concentration 65.4% at 40mg/ml EO then Anise, Caraway, Cinnamon EO (54.1, 45.4 and 6.8 % respectively) at the same concentration. These percentage increased by increasing the time of incubation of EO in the dark with DPPH, that was shown clearly after 120min Cu-EO showed the highest scavenging activity 87.4% then An-EO 72.4%, Ca-EO 59.5 %, Ci-EO 18.2 % at high concentration of EO 40mg/ml . As a result, Cu-EO has a substantially active radical scavenging function relative to An-EO, Ca-EO, Ci-EO. Ascorbic acid was used as a reference here, showing peak activity at very low concentrations Figure (1E). Romeilah *et al.,* recorded that Cu-EO's radical inhibition was 83.59 percent, increasing the essential oil's scavenging activities with the increased concentration of essential oil.[28]De et *al.,* demonstrated that Cu-EOs have high antioxidant activity as a result of the presence of monoterpene alcohols, essential flavours, flavonoids and poly-phenolic.[29]

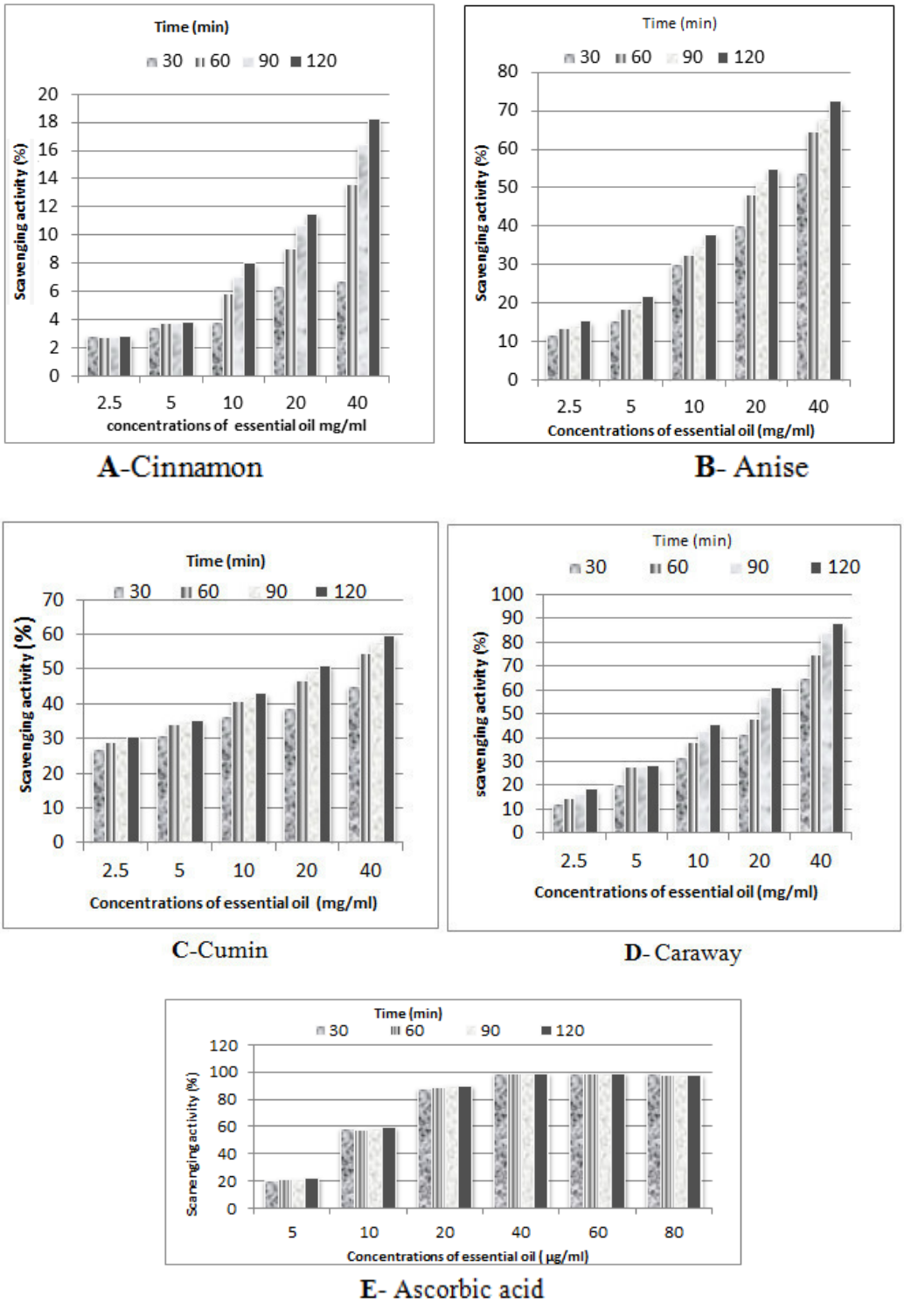


Fig 1. DPPH scavenging activity of spices essential oils and ascorbic acid.

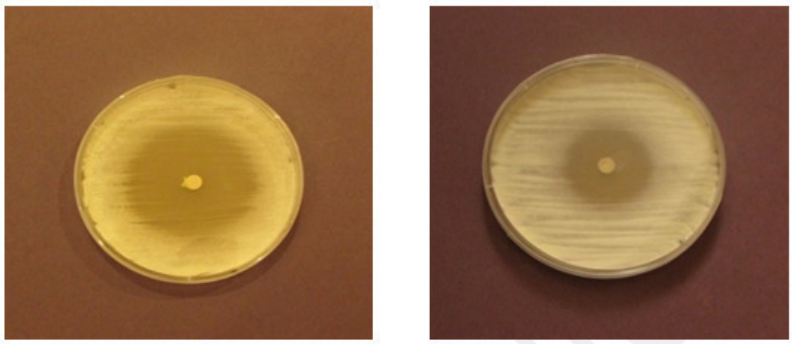
3.3 Antifungal activity

Antifungal activity of EOs of Cinnamon, Anise, Cumin and Caraway against pathogens *A. niger, A. flavus, A. fumigatus, C. krusei* ATCC (6258) and *C. parapsilosis* ATCC (22019) using disc diffusion technique is reported in Table 2. The results of antifungal activity were taken after 7 days of incubation with fungi and 48hr with yeast. The results showed that all tested EOs exhibited antifungal against all the tested pathogens fungi and yeast. The maximum antifungal activity of Ci-EO was shown in the diameter of inhibition zone (55mm) with *A. fumigates.* Mycelium of *A. fumigates* appeared with gray-greenish spores around the inhibition zone. At the same time mycelium of *A. niger* appeared with black spores around the inhibition zone (30mm), while mycelium of *A. flavus* only appeared without spores production around the inhibition zone (35mm).The maximum antifungal activity of An-EO was shown in the diameter of inhibition zone (30 mm) with *A. niger* and *A. fumigatus.* An-EO showed light mycelium only with *A. niger* without spore around the inhibition zone (30mm). *A. fumigatus* mycelium also exhibited without spore around the inhibition zone (30mm) Fig. (2A). *A. flavus* mycelium only appeared without spores production around the inhibition zone (20mm) Fig. (2B).Cu-EO showed complete inhibition of growth (No growth found at all in petri dishes) for all strains of Candida and *A. niger*. Cu-EO also showed antifungal activity with *A. flavus* which mycelium had spore production around the inhibition zone (45mm) and *A. fumigatus* which mycelium didn't have spore around the inhibition zone (20mm).Ca-EO showed antifungal activity with all tested strains where it showed (20 mm) inhibition zone and (45 mm) mycelium only without spores production around the inhibition zone with *A. niger* Fig. (3A).While mycelium of A. *flavus* only appeared without spores around the inhibition zone. At the same time *A. fumigates* mycelium appeared with light spores around the inhibition zone Fig. (3B).

Table 2. Antifungal activity of EOs of Cinnamon, Anise, Cumin, and Caraway against various pathogens fungi using disc diffusion method.

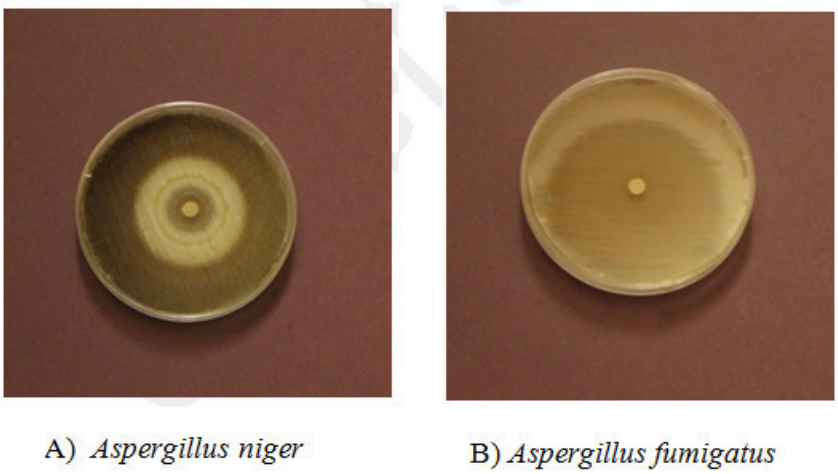
Strain	Inhibition zone (mm)			
	Cinnamon	Anise	Cumin	Caraway
Aspergillus niger	30	30	CI	45
Aspergillus flavus	35	20	45	25
Aspergillus fumigatus	55	30	20	35
Candida krusei ATCC (6258)	45	24	CI	42
Candida parapsilosis ATCC (22019)	45	25	CI	15

CI: complete inhibition



A) *Aspergillus fumigatus* B) *Aspergillus flavus*

Fig 2. Antifungal activities of *Pimpinella anisum (Anise)* essential oil against some pathogenic fungi by using disc diffusion method after 7 days of incubation at 25 C°.



A) *Aspergillus niger* B) *Aspergillus fumigatus*

Fig 3. Antifungal activities *of Carum carvi* (Caraway) essential oil against some pathogenic fungi by using disc diffusion method after 7 days of incubation at 25 C°.

Anti-fungal activity of these EOs may be due to the presence of a phenolic OH group and an aromatic nucleus recognized to be reactive and capable of forming hydrogen bonds with –SH groups in the active sites of focus enzymes, contributing to deactivation of fungal enzymes[30-32]. Cinnamon oil's major constituent is cinnamaldehyde, the compound that contains aldehyde and conjugates double bond outer the ring. Such compound has much greater antifungal activity [33]and may be lead compound for production of antifungal vehicle by regulating chitin synthesis and β-(1, 3)-glucan in molds and yeasts.[34] Yaru*et al.,* investigated the mechanism of how cinnamon oil affects cell morphology, cell membrane, and main enzyme activity through electron microscope scanning (SEM) observations revealed that mycelia morphology alterations of fungi are strikingly shrivelled and collapsed hypha, also flattened empty hyphae, swelled cell wall, disrupted plasma membrane, cytoplasmic matrix leakage. In addition, cinnamon oil inhibited ergosterol biosynthesis significantly damaging the structure of the cell membrane, resulting in intracellular ions, protein leakage and lower absorption at 260 nm. In addition, Ci-EO has influenced the fungal energy metabolism by decreasing the activity of succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) in the process of tricarboxylic acid (TCA).[35] Cu-EO in vitro antifungal activities have been studied against, *C. dubliniensis* ATCC CD60, *C. albicans* ATCC 14053, *C. krusei* ATCC 6258,*C. parapsilosis* ATCC 22019 and *C. glabrata* ATCC 90030. *Cuminum cyminum* oil has widespread antifungal activity against multiple pathogenic species of Candida. The inhibition zone values against the tested species ranged from 7 to 50 mm. Cuminum cyminum oil's best minimal inhibitory concentration (MIC) has been reported against *C. Albicans* as well as *C. dubliniensis* (289 mg / l).[36] Cumin oil antifungal activity was assessed on the growth of 90 fungal isolates in mycelia. Agar-well diffusion method was applied to determine the inhibition of fungal growth at a concentration of 100%. Cu-EO has been highly effective against all tested fungal isolates. Once applied to the solid medium, it was totally prevented mycelia growth of all fungi.[37] Kosalec *et al.,* tested the antifungal efficacy of essential oil of *P. anisum* against, Candida species and confirmed that the EO exhibits a low minimum inhibitory concentration against yeasts in vitro.[38]The anise EO antifungal activity was also identified against *Alternaria alternata*, *A. niger*, and *A. parasiticus*, with *A. parasiticus* being the most widely used fungus. [39] The results obtained in this study showed that EO of *C. carvi* is an effective inhibitor of *Candida krusei* ATCC (6258) growth with (42 mm) inhibition zone. Begum *et al.,* studied *C. carvi* EO influence on a wide variety of fungi and bacteria and suggested that even at low concentrations, the oil can inhibit growth.[40]Siripornvisal *et al.,* illustrated that *Aspergillus, Fusarium* and *Botrytis* growth was inhibited by using EO of *C. carvi* .[41]Antimicrobial effects of *C. carvi* were attributed to the presence of compounds such as carvone and limonene [42] EOs can typically exert their toxic effect on the fungus by compromising the integrity of the fungal membrane, thus inhibiting processes of ion transport and respiration.[43,44]Generally high antifungal activity of the essential oils may suggest a synergistic relationship between their chemical components as for the antimicrobial activity.

4. CONCLUSION

The spices essential oils of Cinnamon, Anise, Cumin and Caraway proved to be a promising source of biomolecules with potential antioxidant and antifungal activity which may find applications in pharmaceutical and food industries. It was possible to conclude that within the current study's experimental conditions.

5. AUTHORS CONTRIBUTION STATEMENT

Dr. Ghada Saber Ibrahim and Dr.Manal Jameel Kiki designed and performed the experiments, wrote the manuscript and reviewed the manuscript. All authors read and approved the final version.

6. CONFLICT OF INTEREST

Conflict of interest declared none.

7. REFERENCES

1. Djilani A, Dicko A. The Therapeutic Benefits of Essential Oils, Nutrition, Well-Being and Health. In: Bouayed J. (Ed.), ISBN: 2012; 978-953-51-0125-3, In Tech, DOI: 10.5772/25344.
2. Ibrahium MI, Abd El-Ghany ME, Ammar MS. Effect of Clove Essential Oil as Antioxidant and Antimicrobial Agent on Cake Shelf Life. World Journal of Dairy & Food Sciences. 2013; 8: 140-146. DOI: 10.5829/idosi.wjdfs.2013.8.2.7633
3. Lewis K, Ausubel FM. Prospects for plant–derived antimicrobials. Nature Biotech.2006; 24:1504–1507; DOI:10.1038/nbt1206-1504
4. Lacaz CS, Porto E, Heins-Vaccari EM, Melo NT. Fungos, actiniomicetos, algas de interessemédico. São Paulo: Sarvier. 1998; 3- 87.
5. Burt S. Essential oils: their antibacterial properties and potential applications in foods. Int. J. Food Microbiol. 2004; 94:223-225. DOI:10.1016/j.ijfoodmicro.2004.03.022
6. Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O. Introduction to Food and Airbone Fungi. Utrecht, The Netherlands: Central bureau voor Schianmel cultures (CBS). 2004; P-389. Available from: https://www.cabdirect.org/cabdirect/abstract/20053187047
7. Kurita N, Miyaji M, Kurane R, Takahara Y, Ichimura K Antifungal activity of components of essential oils. Agriculture and Biological Chemistry. 1981; 45:945–952; DOI:10.1080/00021369.1981.10864635
8. Hajlaoui H, Mighri H, Noumi E, Snoussi M, Trabelsi, N, Ksouri R, Bakhrouf, A. Chemical composition and biological activities of Tunisian *Cuminum cyminum* L. essential oil: a high effectiveness against Vibrio spp. strains. Food Chem. Toxicol. 2010; 48:2186–2192. DOI:10.1016/j.fct.2010.05.044
9. Norman J. The Complete Book on Spices. Doerling Kindersley, London.1999:P-144.
10. Khosravi AR, Shokri H, Minooeianhaghighi M. Inhibition of aflatoxin production and growth of *Aspergillus parasiticus* by *Cuminum cyminum*, Ziziphoraclinopodioides and Nigella sativa essential oils. Foodborne Pathog. Dis. 2011; 8 (12): 1275–1280. DOI:10.1089/fpd.2011.0929
11. Mohammadpour H, Moghimipour E, Rasooli I, Fakoor,M.H., Astaneh, S.A., Moosaie, S.S., Jalili, Z.

Chemical composition and antifungal activity of *Cuminum cyminum* L. essential oil from Alborz mountain against *Aspergillus species*. Jundishapur. J. Nat. Pharm. Prod. 2012; 7 (2): 50–55.
DOI: 10.5812/jjpharma.3445

12. Mortiz CMF, Rall VLM, Saeki MJ, Junior AF. Assessment of antimicrobial activity of cinnamon (*Cinnamomum zeylanicum*) essential oil combined with EDTA and polyethylene glycol in yogurt. ActaScientiarum. 2015; 37(1): 99-104.
DOI: 10.4025/actascitechnol.v37i1.17325
13. Moarefian M, Barzegar M, Sattari M. *Cinnamomum zeylanicum* essential oil as a natural antioxidant and antibacterial in cooked sausage. J Food Biochem. 2013; 37: 62-69.
DOI:10.1111/j.1745-4514.2011.00600.x
14. Usha M, Ragini S, Naqvi SMA. Antibacterial activity of acetone and ethanol extracts of cinnamon (*Cinnamomum zeylanicum*) and ajowan (*Trachys permumammi*) on four food spoilage bacteria. Int Res J Biological Sci. 2012;1(4): 7-11.
15. Fei LU, Yi-cheng D, and Xing-qian YE, Yu-ting D. Antibacterial effect of cinnamon oil combined with thyme or clove oil. AgriSci China. 2011; 10(9): 1482-1487. DOI: 10.1016/S1671-2927(11)60142-9
16. Ayoughi F, Barzegar M, Sahari MA and Naghdibadi M. Chemical compositions of essential oils of *Artemisia dracunculus* L. and *Matricaria chamomile* and evaluation of their antioxidative effect. Journal of Agricultural Science and Technology. 2011; 13: 79-88. Available form:
https://www.researchgate.net/publication/281233289
17. Renaud SC, Gueguen R, Schenker J, d'Houtaud A. Alcohol and mortality in middle-aged men from Eastern France. Epidemiology. 1998;9(2):184–188; PMID: 9504288
18. Temple NJ. Antioxidants and disease: More questions than answers. Nutrition Research. 2000; 20: 449-459. DOI: 10.1016/S0271-5317(00)00138-X.
19. Hae-Ryong P, Eunju P, A-Ram R, Kyung-Im J, Ji-Hwan H, Seung-CheolL. Antioxidant activity of extracts from *Acanthopanax senticosus* .African Journal of Biotechnology . 2006; 5(23):2388-2396. Available form: http://www.academicjournals.org/AJB
20. Leboffe MJ and Pierce BE. A Photographic Atlas for the Microbiology Laboratory, 3rd edition. Morton Publishing Co. ISBN.2005; 0-89582-656-9. Available form: http://www.morton-pub.com/productdetails.cfm
21. Wang M, Xiong YH, Zeng MM, Li HD, Zhang TM and Liang YZ. GC-MS combined with chemometrics for analysis of the components of the essential oils of sweet potato leaves Chromatographia. 2010; 71: 891-897. DOI:10.1365/s10337-010-1561-6
22. Zcan MMO¨ and Chalchat JC. Chemical composition and antifungal effect of anise (*Pimpinella anisum* L.) fruit oil at ripening stage. Annals of Microbiology. 2006; 56(4): 353-358.
DOI:10.1007/BF03175031
23. Hänsel R, Sticher O and Steinegger E. Pharmakognosie Phytopharmazie, 6th ed., Springer-Verlag, Berlin. 1999; pp. 692–695.
DOI: 10.1007/978-3-540-34281-6
24. Li R, Jiang Z. Chemical composition of the essential oil of *Cuminum cyminum* L. from China. Flavour Fragr. J. 2004; 19:311-13.
DOI: 10.1002/ffj.1302
25. Jirovetz L, Buchbauer G, Stoyanova AS, Georgiev EV , Damianova ST. Composition, quality control and antimicrobial activity of the essential oil of cumin (*Cuminum cyminum* L.) seeds from Bulgaria that had been stored for up to 36 years. Int. J. Food Sci. Technol. 2005; 40: 305–310.
DOI: 10.1111/j.1365-2621.2004.00915.x
26. Mohammadpour H, Moghimipour E, Rasooli I, Fakoor M H, Astaneh SA , Moosaie SS , Jalili Z. Chemical composition and antifungal activity of *Cuminum cyminum* L. essential oil from Alborz mountain against Aspergillus species. Jundishapur. J. Nat. Pharm. Prod. 2012; 7 (2): 50–55.Available form: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3941858/pdf/jjnpp-07-50.
27. Khalil N, Mohamed A, Sahar F, Abdel Naser S, Osama S. Chemical composition and antimicrobial activity of the essential oils of selected Apiaceous fruits. Future Journal of Pharmaceutical Sciences. 2018; 4:88-92. DOI:10.1016/j.fjps.2017.10.004
28. Romeilah RM, Fayed SA and Mahmoud GI. Chemical compositions, antiviral and antioxidant activities of seven essential oils. Journal of Applied Sciences Research 2010; 6(1):50-62. Available from: https://www.researchgate.net/profile/Sayed_Fayed/publication/234015257_4-_albhth_alrab/links/0fcfd50e46314e6454000000.pdf
29. De ML, De FV, Fratianni F, Nazzaro F. Chemistry, antioxidant, antibacterial and antifungal activities of volatile oils and their components. Nat Prod Commu 2009; 4(12):1741-50.
DOI: 10.1177%2F1934578X0900401226
30. Farag RS, Daw ZY, Abo-Raya SH. Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. J. Food Sci. 1989; 54: 74–76.
DOI:10.1111/j.1365-2621.1989.tb08571.x
31. Cowan MM. Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews. 1999; 12(4): 564–582. DOI: 10.1128/CMR.12.4.564.
32. Velluti A , Sanchis V, Ramos AJ, Egido J, Mar1n S. Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and fumonisin B1 production by *Fusarium proliferatum* in maize grain. J. Food Microbiol. 2003; 89: 145–154. DOI: 10.1016/s0168-1605(03)00116-8.
33. Wang SY, Chen PF, Chang ST. Antifungal activities of essential oils and their constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) leaves against wood decay fungi Bioresour. Technol. 2005; 96: 813–818. DOI: 10.1016/j.biortech.2004.07.010
34. Bang KH, Lee DW, Park HM, Rhee YH. Inhibition of fungal cell wall synthesizing enzymes by transcinnamaldehyde. Biosci. Biotech. Biochem. 2000; 64: 1061–1063.
DOI: 10.1271/bbb.64.1061
35. Yaru Li Y, Ying N, Zhou L, Li S, Tang X, Ding Y, Li S. The possible mechanism of antifungal activity of cinnamon oil against *Rhizopus nigricans*. Journal of Chemical and Pharmaceutical Research. 2014; 6(5):12-20.Available form: https://pdfs.semanticscholar.org/f7cc/b8eb4a2a7bacd8503ecbf2272a9bb0c54738.
36. Naeini A, Naderi NJ, Shokri H. Analysis and in vitro anti-Candida antifungal activity of *Cuminum cyminum* and *Salvadora persica* herbs extracts against pathogenic

Candida strains. J Mycol Med 2014; 24(1): 13-18. DOI:10.1016/j.mycmed.2013.09.006.

37. El-Said AHM, El-Hady G. Antifungal activities of *Cuminum cyminum* and *Pimpinella anisum* essential oils. Int. J. Curr. Microbiol. App Sci 2014; 3(3): 937-944. Available form: https://www.ijcmas.com/vol-3-3/A.H.M.El-Said%20and%20El-Hady%20Goder.
38. Kosalec I, Pepeljnjak S, Kustrak D. Antifungal activity of fluid extract and essential oil from anise fruits (*Pimpinella anisum* L., Apiaceae). Actapharmaceutica (Zagreb, Croatia). 2005; 55(4): 377-385. PMid: 16375827 Available form: http://acta.pharmaceutica.farmaceut.org/37705.pdf
39. Begum J, Islam Bhuiyan M, Chowdhury J, NuzmuHoque M, Nural Anwar M. Antimicrobial activity of essential oil from seeds of *Carum carvi* and its composition. Bangladesh J. Microbiol. 2008; 25 (2):85-89. DOI:10.3329/bjm.v25i2.4867
40. Gholami H, Mokhtari M, Mohammadian M, RuhollahMontazeri R. Antifungal effect of *Bunim persicum* essential oil on floconazol resistance clinical isolate of *Candida galbrata*. IICM. 2012; 536-540.
41. Siripornvisal S, Thawornluk P, Rungprom W. Screening for antifungal activity and active components of crude extracts from 6 Umbelliferae.WarasanWitthayasatKaset. 2011; 42(2): 361-364. Available from: http://agris.fao.org/agris-search/search.do?recordID=TH2016001869
42. Iacobellis N, Cantore P, Capasso F, Felice S, Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* essential oils. J. Agric. Food Chem. 2005; 53:57-61. DOI: 10.1021/jf0487351.
43. Knoblock K, Pauli A, Iberl B, Weis N, Weigand H. Antibacterial activity and antifungal properties of essential oil components. Journal of Essential Oil Research. 1988; 1: 119-128. DOI:10.1080/10412905.1989.9697767