Journal of Applied and Natural Science
16(4), 1679 - 1689 (2024)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Chemical composition of essential oils of some common culinary spices and their antimicrobial activities

Neetu Singh
Department of Botany, Maharshi Dayanand University, Rohtak-124001 (Haryana) India
Surender Singh Yadav
Department of Botany, Maharshi Dayanand University, Rohtak-124001 (Haryana) India
Balasubramaniam Narashiman
Dept. of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak-124001 (Haryana) India

*Corresponding author. E-mail: ssyadavindia@gmail.com

Article Info
https://doi.org/10.31018/jans.v16i4.5917
Received: July 4, 2024
Revised: November 24, 2024
Accepted: November 29, 2024

How to Cite
Singh, N. *et al.* (2024). Chemical composition of essential oils of some common culinary spices and their antimicrobial activities. *Journal of Applied and Natural Science*, 16(4), 1679 - 1689. https://doi.org/10.31018/jans.v16i4.5917

Abstract
Antimicrobial resistance is one of the top 10 global threats to public health. Natural products can be effectively used to prevent antimicrobial resistance. Spices are highly used food commodities. Their essential oils (EOs) possess excellent antimicrobial potential. Therefore, the present study aimed to extract oil from three commonly used spices and assess their antimicrobial activity.The EOs were extracted from cinnamon (*Cinnamomum verum* J. Persel), cumin (*Cuminum cyminum* L.) and trigonella (*Trigonella foenum-graecum* L.) and were characterized by GC-MS. Antimicrobial activities were screened against two Gram +ve bacterial strains: *Staphylococcus aureus* (MTCC No. 737), *Bacillus subtilis* (MTCC No. 441); two Gram-ve bacterial strains, *Escherichia coli* (MTCC No. 443), *Pseudomonas aeruginosa* (MTCC No. 1688); and two fungal strains: *Aspergillus oryzae* (NCIM1008) and *Fusarium oxysporum* (MDU-4) via disc diffusion and broth dilution assays.The highest yield of EOs was obtained with *trigonella* (4.35 %), followed by cinnamon (2.75%) and cumin (1.53%). GC-MS analysis confirmed the presence of various phytocompounds in different EOs. Antimicrobial results showed that cinnamon EO was the most active with the maximum zone of inhibition of 15.3±0.08 mm, 11.3±0.25 mm, and 13.1±0.14 mm against *E. coli*, *P. aeruginosa*, and *F. oxysporum*, respectively. Cumin EOhas also shown promising antimicrobial activity with a zone of inhibition of 13.0±0.09 mm, 10.2±0.20 mm, and 11.4±0.08 mm against *E. coli*, *P. aeruginosa*, and *F. oxysporum*. *E. coli* and *F. oxysporum* were the most sensitive microbial strains.Cinnamon EO exhibited the highest antimicrobial activity and, therefore, can be used as a natural antimicrobial herb after clinical trials.

Keywords: Antimicrobial, Essential oils, Food, Gas chromatography-mass spectrometry (GC-MS), Natural products, Spices

INTRODUCTION

Antimicrobial resistance is one of the top 10 global threats to public health. It occurs when microbes (bacteria, viruses, fungi and parasites) do not respond against applied drugs. In appropriate use of antibiotics, poor hygiene and some natural factors may be the leading causes of increased incidents of antimicrobial resistance. Since the last century, antibiotic resistance has failed most medical breakthroughs (Aslam *et al.,* 2018). It is spreading at an alarming rate and transforming many curable infectious diseases into untreatable ones. According to “Antimicrobial Resistance: Global Report on Surveillance (2014)” antimicrobial resistance can spread all over the map. It can exert its negative influence on any person of any age and any nation. Nearly five million deathswere reported due to antimicrobial resistance in 2019 (https://www.who.int/). It has also been estimated that by 2050, antimicrobial resistance will result in a death rate of 10 million/year (Cresti *et al.,* 2022). Therefore, a multisectoral and multidisciplinary approach is needed to manage this havoc (Joshi *et al.,* 2018).

In its report, World Health Organization (WHO 2023) emphasized the major causes of antibiotic resistance and the global policy framework to combat this emerging health problem. WHO has also scheduled a high-level United Nations General Assembly meeting in September 2024 to make bold decisions and commitments to resolve this problem (https://www.who.int/).

Other measures are also needed besides WHO initiatives because it is impossible to fight antimicrobial resistance with a single response. Synthetic antimicrobials are also evolving as powerful weapons but are associated with several adverse effects. Their success rate remains doubtful (Mittal *et al.,* 2019). For instance, SET-M33 is a non-natural antimicrobial peptide that leads to a dose-dependent increase in creatinine, urea level and degeneration and regeneration in the kidney (Cresti *et al.,* 2022). They also adversely impact the economy, resources and life expectancy. Everyone looks forward to curing antimicrobial resistance with natural medicines (Eldin *et al.,* 2023; Rao *et al.,* 2021). Natural products are biodegradable in nature and possess novel sites of action (Batish *et al.,* 2007). Medicinal plants, herbs and spices have been used for centuries as natural medicines for curing microbial infections in humans and animals (Mittal *et al.,* 2019).

Spices are the culinary herbs and dried plant parts that improve food items' taste, flavor, color and aroma (Singh and Yadav, 2022a; Vázquez-Fresno *et al.,* 2019). They are also used for maintaining good health, treating routine maladies and boosting immunity (Ogbunugafor *et al.,* 2017). They also act as a good antimicrobial agent and have wide applications in pharmaceutical industries (Yousefi *et al.,* 2019). Researchers are exploring the therapeutic potential of spices across the globe.

India is popularly considered as the goldmine of spices. Over a hundred medicinal plants are used as spices for culinary and therapeutic purposes (Singh and Yadav, 2022b).The common culinary spices *viz. Cinnamomum verum*, *Cuminum cyminum* and *Trigonella foenum-graecum* are widely distributed. *Ci. verum* J. Persel is commonly known as cinnamon and belongs to the family Lauraceae. Its bark is of great culinary and therapeutic importance.The genus comprises almost 250 species and is widely distributed in India, China and Australia. Traditionally, it is used to cure asthma, bronchitis, cardiovascular diseases and others (Firmino *et al.,* 2018). It is also used to cure microbial infections, oxidative stress, inflammation, diabetes and cancer. Though these therapeutic properties of cinnamon are cumulative effects of its diverse phytocompounds but, they are mainly attributed to its major phytocompound cinnamaldehyde i.e., majorly constituted in cinnamon EO (Prabuseenivasan *et al.,* 2006; Singh *et al.,* 2021a). *Cu. cyminum* L. is a small-sized herb i.e. commonly known as cumin. It belongs to the family Apiaceae. It is frequently cultivated in India, Egypt, China, the Middle East, and other Mediterranean countries. The plant has several folkloric and medicinal values. It can be effectively used as a gastric stimulator, antimicrobial, antioxidant, antidiabetic and anticancer agent (Mnif and Aifa, 2015).I ts different phytocompounds render the diverse therapeutic effects of cumin. Among different bioactive compounds, cuminaldehyde was the principal phytoconstituent (Singh *et al.,* 2021b).

T. foenum-graecum L. is also known as fenugreek. It belongs to the family Fabaceae. The plant has a global distribution. India is the largest producer, followed by China, Pakistan, Iran, Afghanistan, Russia, Australia, Canada, USA and Europe. In these countries, this plant is traditionally used in boosting immunity and curing diabetes, inflammation, hypercholesterolemia, digestive and reproductive problems (Goyal *et al.,* 2016). These traditional uses of *trigonella* also endorsed its multiple pharmacological effects. *Trigonella* seed oil has numerous pharmacological effects, which help to cure many maladies in humans and animals (Singh *et al.,* 2022). Keeping this in mind, the present study aimed to explore the chemical composition and the antimicrobial potential of three commonly used dietary spices, viz. cinnamon (*Ci. verum*), cumin (*Cu. cyminum*), and trigonella (*T. foenum-graecum*), against selected bacterial and fungal strains.

MATERIALS AND METHODS

Chemicals used

The analytical-grade chemicals were procured from Sigma-Aldrich and Himedia.

Test materials

Cinnamon (bark), cumin (seeds) and trigonella (seeds) were procured from the local market of Rohtak, Haryana (India). These spices were selected based on various factors like their dietary fiber, protein and fat content which are comparatively rich in fat, protein and dietary fibers (https://www.usda.gov/).

Extraction of plant materials

Cinnamon (bark) and cumin (seed) powder were extracted using 100 gm of both spice powdersusingthe Clevenger apparatus. The powder was dissolved in 500 mL of distilled water. Extractions were performed at 100 ˚C for 5-10 minutes, thereafter, the temperature was set at 40-60 ˚C (until no more EOs were obtained). EOs were collected, dried with anhydrous sodium sulfate and stored at 4 ˚C until analysis (Pingret *et al.,* 2014). *Trigonella* (seed powder) was extracted using the Soxhlet apparatus. A thimble containing 50 gm of seed powder was prepared and extracted with petroleum ether. The cycle was run at 40-60 ˚C (until no more EO was extracted). The obtained EO was left overnight for the evaporation of the solvent. The water droplet of EO was removed with anhydrous sodium sulfate. The dried EO was collected and stored at 4 ˚C for further analysis (Akbari *et al.,* 2019).

Quantification of essential oils (EOs)

The EOs extracted using different extraction methods

were quantified by following the equation developed by Akbari *et al.* (2019):

$$\% \text{ Yield of EO} = \frac{\text{Mass of EO obtained (gm)}}{\text{Weight of the sample taken (gm)}} \text{ x } 100$$

Eq.1

Gas chromatography-mass spectrometry (GC-MS) and compound identification

GC-MS analysis was performed using 394143602-GC Single Quadruple Mass Spectrometer SCION 436 GC; Fill Scan SIM, with CP-739651-CP8410 Liquid Auto Sample. The operation conditions were as follows: vector gas, helium; flow rate, 1 mL/min.; column temperature, 100 ˚C; injected volume, 1 µL. Each peak in the chromatogram corresponds to a particular compound. Peak areas were used to quantify the relative amounts of individual compounds.Compounds were identified with the help of commercial and mass spectra libraries combined with the computer. The mass spectra data was compared with the GC retention indices (Yang *et al.,* 2021).

Assessment of antimicrobial activity

Disc diffusion assay

The antimicrobial activity was evaluated against two Gram-positive *Staphylococcus aureus* (MTCC No. 737), *Bacillus subtilis* (MTCC No. 441); two Gram -ve bacterial strains: *Escherichia coli* (MTCC No. 443), *Pseudomonas aeruginosa* (MTCC No. 1688); and two fungal strains: *Aspergillus oryzae* (NCIM1008) and *Fusarium oxysporum* (MDU-4).The microbial strains were procured from the Department of Microbiology, M.D.University. Rohtak. The activity was performed using 37 ˚C overnight bacterial culture in nutrient broth and 30±5 ˚C (2-3 days) fungal culture in potato dextrose broth. The microbial concentrations were adjusted at 8 x 10^8 cells/mL at 600 nm. A hundred microliter microbial suspension was spread over the agar plates. The culture was evenly spread with the help of a sterile spreader to achieve uniform microbial growth over plates. EOs were diluted in dimethyl sulfoxide (DMSO) to get a stock solution of 10 mg/mL. Under aseptic conditions of laminar airflow, the sterile discs (6 mm diameter) were infused with 30 µL of different concentrations (100, 50, 25, 12.5, 6.25 mg/mL) of different EOs. Paper discs impregnated with DMSO were placed on seeded plates as the standard. Ciprofloxacin and fluconazole discs were used as a reference control for antibacterial and antifungal activity, respectively. All petri plates were sealed with parafilm to avoid sample evaporation and external contamination. The plates were incubated at 37 ˚C overnight for bacterial and 30±5 ˚C (48 hours) for fungal cultures. After incubation, the zone of inhibition was measured with a caliper. Experiments were performed in triplicates and the data were presented as mean values forof triplicates (Saki *et al.,* 2020).

Minimum Inhibitory Concentration (MIC) assay

The broth dilution method was employed for *in-vitro* MIC (mg/mL) estimation in which 20 µL aliquot (prepared in 0.85 % of NaCl) was added to test tubes. Inoculated test tubes were incubated for a period of 12-18 hours at 37±2 ˚C and 48-60 hours at 30±5 ˚C for bacterial and fungal strains, respectively. Experiments were carried out in triplicates. The inhibition of microbial growth in the test tubes containing EOs was observed by comparing it with microbial growth in the blank control test tube. The EOs concentration without visible turbidity in test tubes was considered the best MIC for respective microbes (Saki *et al.,* 2020).

Minimum bactericidal concentration and minimum fungal concentration

The freshly prepared agar plates were spread with 10 µL of MIC culture and then incubated for 12-18 hours at 37±2 ˚C and 30±5 ˚C for bacterial and fungal strains, respectively. The microbial growth was visually observed (Saki *et al.,* 2020).

RESULTS

Quantification of essential oils (Eos)

The yields of EOs extracted from different spices are mentioned in Table 1.

Maximum yield was obtained with Trigonella EO followed by cinnamon and cumin. Therefore, it can be inferred that the Soxhlet apparatus yielded a higher EO than the Clevenger apparatus.

Composition analysis of essential oils

GC-MS analysis of different EOs was done to identify their phytochemical composition. The compounds were identified by comparing the analytical libraries' retention indices and mass spectra. The GC-MS chromatograms of different EOs are depicted in Fig. 1a, 1b, and 1c. Compounds identified from EOs of cinnamon, cum-

Table 1. Quantification of different essential oils of selected spices

Spices	Mass (gm)	Volume (mL)	Specific density (g/mL)	% Yield
Cinnamon	12.36	12	1.03	2.75
Cumin	6.9	7.5	0.92	1.53
Trigonella	19.6	20	0.98	4.35

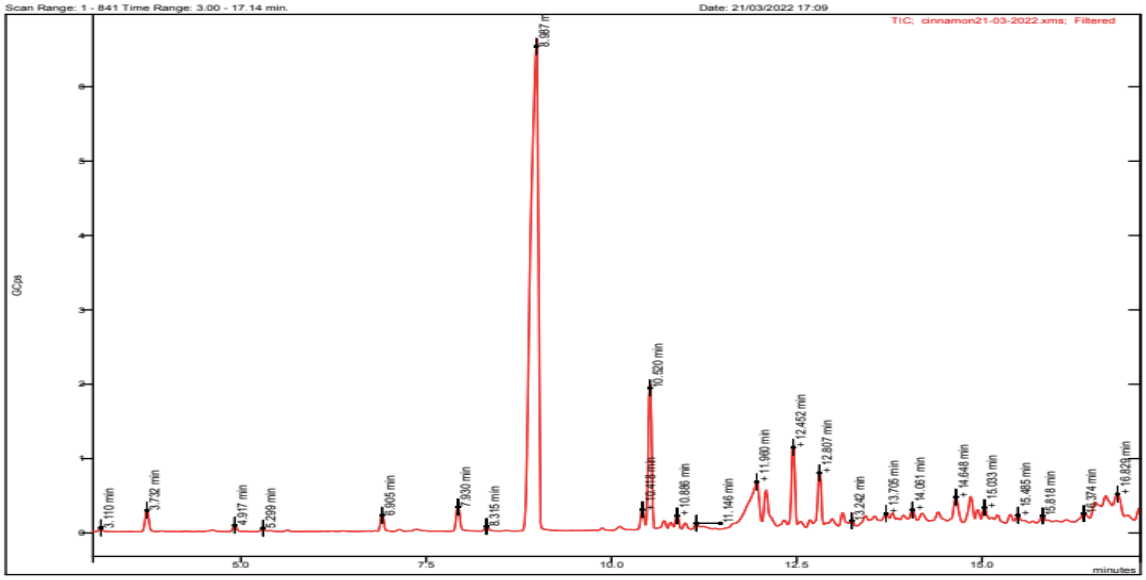


Fig. 1a. *Gas Chromatography-Mass Spectrometry chromatogram of cinnamon essential oil*

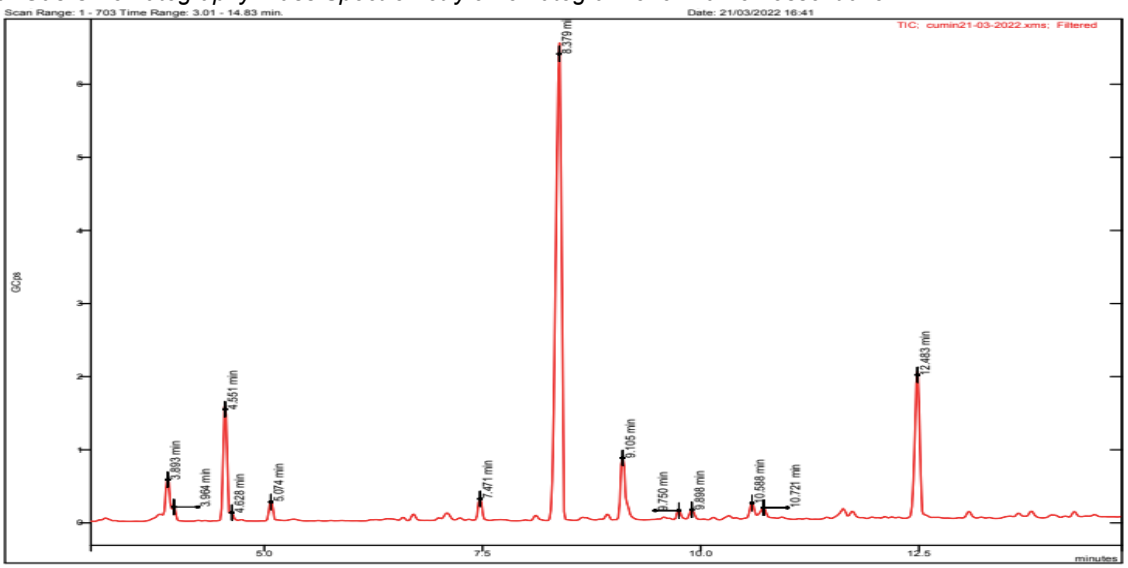


Fig. 1b. *Gass Chromatography-Mass Spectrometry chromatogram of cumin essential oil*

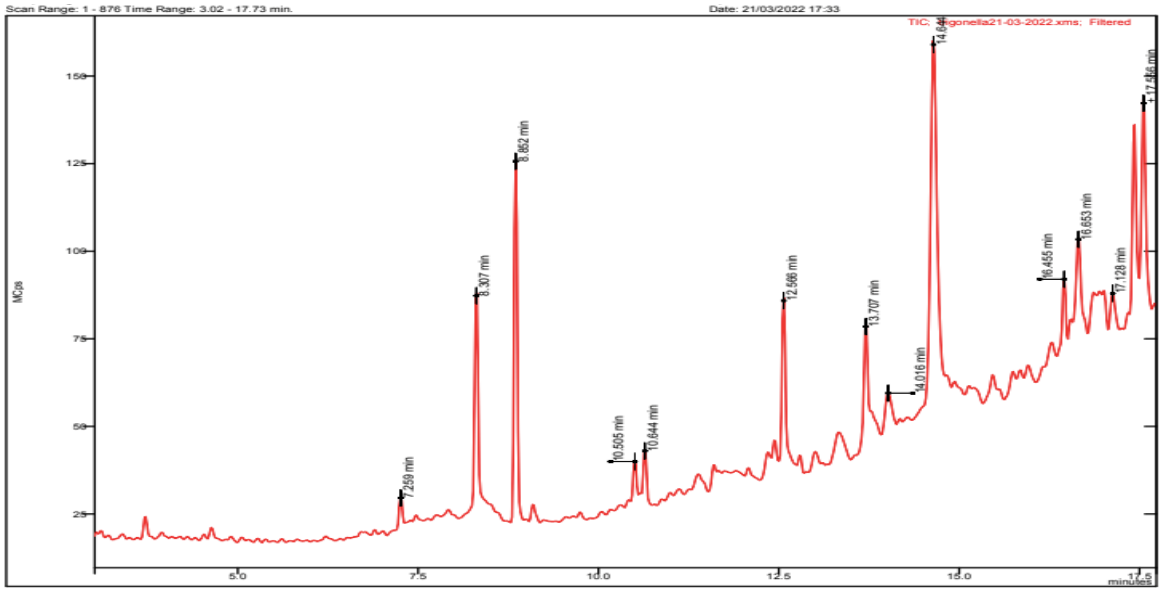
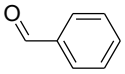
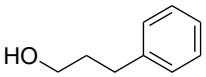
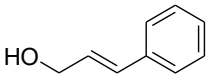
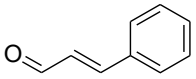
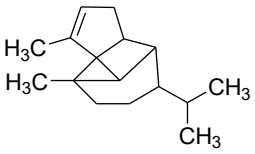
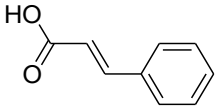
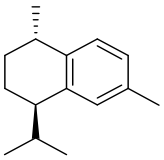
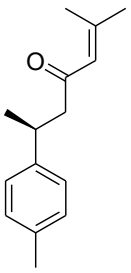


Fig. 1c. *Gass Chromatography-Mass Spectrometry chromatogram of trigonella seed oil*

Table 2a. Chemical composition of cinnamon essential oil

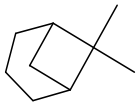
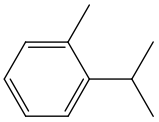
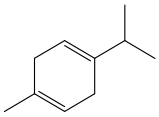
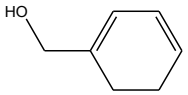
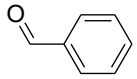
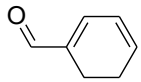
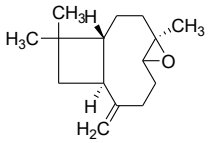
Retention time	Compound name	Area	Probability	Molecular formula	Molecular weight (g/mol)	Structure of compound
3.732	Benzaldehyde	1.035	34.52	C_7H_6O	106.12	
6.905	Benzenepropanol	0.747	74.20	$C_9H_{12}O$	136.19	
7.930	2-Propenol, 3-phenyl	1.244	22.25	$C_9H_{10}O$	134.18	
8.987	Cinnamaldehyde	48.55	37.89	C_9H_8O	132.16	
10.52	α-Copaene	6.479	51.71	$C_{15}H_{24}$	204.35	
11.96	2-Propenoic acid, 3-phenyl	6.812	50.16	$C_9H_8O_2$	148.16	
12.80	trans-Calamene	2.769	2.769	$C_{15}H_{22}$	202.33	
14.64	ar-Tumerene	1.729	1.729	$C_{15}H_{20}O$	216.32	

in and trigonella are depicted in Table 2a, 2b and 2c, respectively.

Antimicrobial activity

The antimicrobial activities of cinnamon, cumin and trigonella are summarized in Tables 3a, 3b and 3c, respectively. The MIC, MBC and MFC activities of different EOs are shown in Figure 2a-2d. Different EOs have shown varying magnitudes of their antimicrobial potential. The zone of inhibition of more than seven mm was considered a good zone of inhibition. Most of the microbial strains were sensitive to EOs. All EOs were active against all strains, but maximum sensitivity was observed for *E. coli* followed by *P. aeruginosa*. In the case of fungal strains, *F. oxysporum* showed better activity than*A. oryzae*. Among different EOs, cinnamon EO was most active against the tested microbial strains. Cumin EO has also shown good antimicrobial activity;

Table 2b. Chemical composition of cumin essential oil

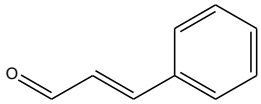
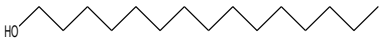
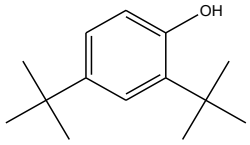
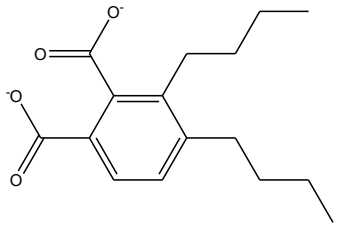
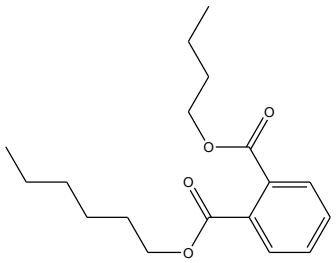
Retention time	Compound name	Area	Probability	Molecular formula	Molecular weight (g/ mol)	Structure of compound
3.893	Bicyclo-heptane 6,6-dimethyl	3.105	49.28	C_9H_{16}	124.22	
4.551	o-Cymene	10.090	18.15	$C_{10}H_{14}$	134.22	
5.074	γ-Terpinene	1.661	28.11	$C_{10}H_{16}$	136.23	
7.471	1,3-cyclohexadiene 1-methanol	1.792	67.55	C_7H_8O	110.15	
8.379	Benzaldehyde	56.268	35.10	C_7H_6O	106.12	
9.105	1,3-cyclohexadiene 1-carboxyaldehyde	6.637	60.18	C_7H_8O	108.14	
12.483	Caryophylleneoxide	15.004	31.58	$C_{15}H_{24}O$	220.35	

no significant antimicrobial activity was observed with trigonella EO. There was no inhibition of growth with the vehicle control i.e. DMSO.

DISCUSSION

Antimicrobial resistance is emerging as a burning public health problem. The constant prevalence of microbial resistance in recent decades has accelerated the use of natural medicines (Eldin *et al.,* 2023). Herbs, spices and medicinal plants can act effectively against antibiotic-resistant microbes (Yu*et al.,* 2020). They also possess countless approaches to the well-being of all living creatures. They have been used not only for food, cosmetics, and pharmaceutical purposes as alternative and natural medicines since antiquity (Yousefi *et al.,* 2019). Spices are a rich reservoir of essential oils that have high antimicrobial efficacy. The multidimensional role of spices EOs in traditional healthcare formulation has necessitated the scientific investigation of these plants. The antimicrobial potential of spice EOs is well-reported. In the present study, the *invitro* antimicrobial screening of EOs has also demonstrated the antimicrobial efficacy of EOs against a panel of microbial strains. However, different EOs have shown different magnitudes of antimicrobial effectiveness. Previously published documents also reported strongand medium antimicrobial effectiveness of cinnamon EOs against drug -resistant bacteria, i.e., methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus faecium*, *Acinetobacter baumannii*, *P. aeruginosa* and *E. coli* (Saki *et al.,* 2020). In the present study, cinnamon EO showed the maximum antimicrobial ZI and MIC (Table 3 and Figure 2, respectively). The maximum activity of cinnamon oil was observed against *E. coli* with a ZI of 15.3±0.08 mm (Table 3b). Maximum antifungal activity was also observed with cinnamon EO. The maximum antifungal ZI of cinnamon EO was 13.1±0.14 mm against *F. oxysporum* (Table 3c). Several studies reported that cinnamon EO has shown strong inhibition against several pathogens (Paudel *et al.,* 2019). Hu *et al.* (2019) studied the antifungal activity of seven EOs against three fungal strains *viz. A. oryzae*, *A. niger* and *A. ochraceus*. They found that cinnamon EO had the maximum antifungal activity against all fungal strains. The maximum growth inhibition efficacy of cinnamon EO was attributed to its biologically active volatile compounds. The present study also reported numerous phytoconstituents in different EOs. Therefore, in the present study, GC-MS was employed to confirm the

Table 2c. Chemical composition of *Trigonella* seed oil

Retention time	Compound name	Area	Prob-ability	Molecular formula	Molecular weight(g/mol)	Structure of compound
8.307	Benzaldehyde 4-(1-methylethyl)	9.074	35.39	NR	NR	NR
8.852	Cinnamaldehyde	13.518	42.87	C_9H_8O	132.16	
10.644	n-Pentadecanol	2.345	4.62	$C_{15}H_{32}O$	228.41	
12.566	Phenol, 2,4-bis (1,1-dimethyl ethyl)	6.785	46.70	$C_{14}H_{22}O$	206.32	
13.707	Aspidospermidin-17-ol, 1-acethyl-19,21-epoxy-15,16-dimethoxy	7.417	25.85	NR	NR	NR
14.644	Dibutyl.pthalate	24.873	15.60	$C_{16}H_{20}O_4^{2-}$	276.33	
16.653	Murolan-3,9 (11) diene-10-peroxy	7.407	10.33	NR	NR	NR
17.428	Pthalic acid butyl hexyl ester	8.315	8.23	$C_{18}H_{26}O_4$	306.4	
17.556	i-Proxy 7,10,13,16,19-docosapent	10.203	9.29	NR	NR	NR

*NR = Not reported

presence of various phytocompounds of EOs. GC-MS analysis revealed that cinnamaldehyde was the major phytoconstituent of cinnamon EO.

The predominant intensity of cinnamaldehyde in cinnamon EO agreed with the study by Singh *et al.* (2021a). Cinnamaldehyde is a natural product, and its consumption helps combat various health ailments (Singh and Yadav, 2024). Though some studies have also reported some toxic effects of cinnamaldehyde, it is not harmful when consumed in food products (Muhoza *et al.,* 2023). Cumin EO also exhibits good antimicrobial potential. It has increased due to cumin EO concentration (Behbahani *et al.,* 2019). The present research has also shown comparable antimicrobial activity of cumin EO against both bacterial and fungal strains. Singh *et al.* (2024) also reported the antimicrobial activity of trigonella EO against different microbial strains. In the present study, trigonella EOs showed poor activity compared to other EOs. The trigonella EO was extracted from the Soxhlet apparatus, which produces qualitatively low-grade EO. This might be the reason behind the poor activity of trigonella EO (Fagbemi *et al.,* 2021). The high efficacy of the spices as antimicrobials may be attributed to the hydrophobic nature of their essential oils. The hydrophobic nature of their essential oils facilitates the penetration of microbial cells. In microbial cells, EOs lead to the partition of lipids from the cell membranes. The disturbed cell structure becomes

Table 3a. Zone of inhibition (mm) of test compounds against *Staphylococcus aureus* and *Bacillus subtilis*[a]

Test compound	Zone of inhibition (mm)									
	Staphylococcus aureus					*Bacillus subtilis*				
	100 %	50 %	25 %	12.5 %	6.25 %	100 %	50 %	25 %	12.5 %	6.25 %
Cinnamon EO	10.9±1.05	8.9±0.29	8.0±0.54	7.9±0.12	6.2±0.04	10.8±0.16	7.1±0.19	6.2±0.14	6.1±0.08	6.0±0.04
Cumin EO	10.1±0.12	7.2±0.08	NA	NA	NA	10.1±0.12	10.1±0.24	7.1±0.08	7.0±0.04	6.2±0.12
Trigonella EO	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

[a] Measurement in triplicate

Table 3b. Zone of inhibition (mm) of test compounds against *Escherichia coli* and *Pseudomonas aeruginosa*[a]

Test compound	Zone of inhibition (mm)									
	Escherichia coli					*Psuedomonas aeruginosa*				
	100 %	50 %	25 %	12.5 %	6.25 %	100 %	50 %	25 %	12.5 %	6.25 %
Cinnamon EO	15.3±0.08	14.2±0.17	12.0±0.05	12.1±0.08	10.0±0.09	11.3±0.25	10.3±0.20	10.2±0.17	10.1±0.19	8.0±0.20
Cumin EO	13.0±0.09	12.2±0.08	11.2±0.17	10.2±0.16	9.2±0.20	10.2±0.20	9.1±0.14	NA	NA	NA
Trigonella EO	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

[a] Measurement in triplicate

Table 3c. Zone of inhibition (mm) of test compounds against *Aspergillus oryzae* and *Fusarium oxysporum*[a]

Test compound	Zone of inhibition (mm)									
	Aspergillus oryzae					*Fusarium oxysporum*				
	100 %	50 %	25 %	12.5 %	6.25 %	100 %	50 %	25 %	12.5 %	6.25 %
Cinnamon EO	12.1±0.12	10.2±0.16	9.1±0.14	8.9±0.09	8.1±0.12	13.1±0.14	12.0±0.05	11.2±0.17	10.0±0.20	7.4±0.08
Cumin EO	10.3±0.12	11.1±0.08	10.2±0.05	10.0±0.05	9.9±0.08	11.4±0.08	10.3±0.20	10.1±0.08	9.0±0.20	8.1±0.12
Trigonella EO	NA	NA	NA	NA	NA	9.1±0.16	8.0±0.24	7.7±0.20	7.4±0.12	6.6±0.14

[a] Measurement in triplicate

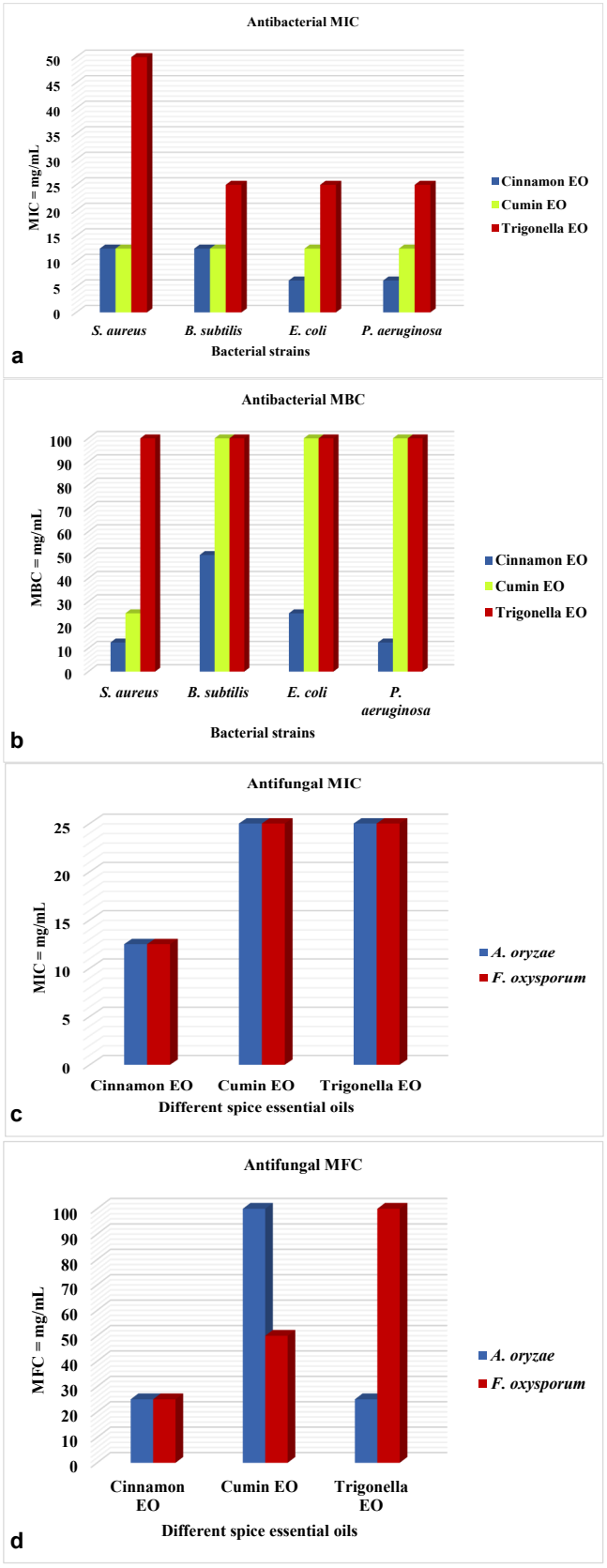


Fig. 2. *Broth dilution results (a) antibacterial minimum inhibitory concentration (MIC), (b) antibacterial minimum bactericidal concentration (MBC), (c) antifungal minimum inhibitory concentration (MIC), and (d) antifungal minimum fungicidal concentration (MFC)*

more permeable to the EOs. This will lead to extensive leakage of critical molecules and ions from the cell. The EOs disturb the cell membrane integrity and ultimately induce cell death (Behbahani *et al.,* 2019). Quorum sensing plays an integral role in the progression of microbial density. It consists of stimuli and responses that regulate virulence factors and biofilm formation (Firmino *et al.,* 2018). EOs of spices disturb the quorum -sensing process and ultimately hinder the bacterial colony. The anti-quorum sensing effect ofcinnamon EO was realized on *P. aeruginosa* (Kalia *et al.,* 2015). Prabuseenivasan *et al.* (2006) evaluated the antimicrobial activity of EOs of 21 medicinal plants. Out of 21 EOs, 19 EOs have shown antibacterial activity. Cinnamon, clove, geranium, lemon, lime, orange and rosemary oils have shown a noticeable antibacterial effect. The maximum activity was observed with cinnamon EO. They reported that Gram+ve bacterial strains (*B. subtilis* and *S. aureus*) were resistant to the spice EO, whereas Gram-ve bacterial strains (*E. coli*, *Klebsiella pneumoniae*, *P. aeruginosa* and *Proteus vulgaris*) were sensitive to the EOs. Several other studies have also shown that EO is more effective against Gram-ve bacterial strains (Prakash *et al.,* 2021). The present study also observed that Gram-ve bacterial strains (*E. coli* and *P. aeruginosa*) were more sensitive to EOs than Gram+ve bacterial cells (*S. aureus* and *B. subtilis*). The reason might be due to the different penetration abilities of EOs into microbial cells. The difference in EO penetration ability arises due to the difference in the composition of the cell walls of Gram+ve and Gram-ve bacterial strains. The present study also highlighted that the oil extracted from the Soxhlet apparatus has shown poorer activity than the oil extracted through the Clevenger apparatus. The present study clearly defines the different extents of antimicrobial activity and the probable reason behind their different activity compared to the previously published studies.

Conclusion

Spices have excellent therapeutic properties that are mainly rendered by their active compounds. These phytocompounds are mainly concentrated in their EOs. Therefore, in the present study, the essential oils were extracted from the bark of *Cinnamomum verum* (cinnamon) and seeds of *Cuminum cyminum* (cumin) and *Trigonella foenum-graecum* (trigonella). The cinnamon bark EO was observed to have the highest antimicrobial potential (against bacterial and fungal strains), followed by cumin EO, which had also possessed moderate antimicrobial activity compared to trigonella EO, but it was lower than cinnamon EO. The maximum antibacterial activity was observed against *Escherichia coli* , whereas the maximum antifungal activity was ob-

tained against *Fusarium oxysporum*. The highest antimicrobial activity of cinnamon EO was assigned to its active principal constituent cinnamaldehyde (confirmed by GC-MS). This cost-effective antimicrobial supplement can be used in developing countries to develop new antimicrobial agents. Further *in vivo* and clinical trials are needed to explore the full efficacy of cinnamon EO.

ACKNOWLEDGEMENTS

Financial assistance from the Council for Scientific and Industrial Research (CSIR), New Delhi and Science and Engineering Research Board-Department of Science and Technology (SERB-DST), Government of India, New Delhi, Fund for Improvement of S&T Infrastructure in Universities & Higher Educational Institutions (FIST), Department of Science and Technology, Govt. of India, New Delhi, Haryana State Council for Science Innovation & Technology (HSCSIT), Panchkula are thankfully acknowledged.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Akbari, S., Abdurahman, N.H., Yunus, R.M., Alara, O.R. & Abayomi, O.O. (2019). Extraction, characterization and antioxidant activity of fenugreek (*Trigonella-Foenum Graecum*) seed oil. *Materials Sciences for Energy Technologies,* 2(2) 349-355. https://doi.org/10.1016/j.mset.2018.12.001.
2. Aslam, B., Wang, W., Arshad, M.I., Khurshid, M., Muzammil, S., Rasool, M.H., Nisar, M. A., Alvi, R.F., Aslam, M.A., Qamar, M.U., Salamat, M.K.F. & Baloch, Z. (2018). Antibiotic resistance: a rundown of a global crisis. *Infection and Drug Resistance,* 1645-1658. https://doi.org/10.2147/IDR.S173867.
3. Batish, D. R., Pal Singh, H., Kohli, R. K., Kaur, S., Saxena, D. B. & Yadav, S. (2007). Assessment of phytotoxicity of parthenin. *Zeitschrift für Naturforschung C*, 62, 367-372. https://doi.org/10.1515/znc-2007-5-609.
4. Behbahani, B.A., Noshad, M. & Falah, F. (2019). Cumin essential oil: Phytochemical analysis, antimicrobial activity and investigation of its mechanism of action through scanning electron microscopy. *Microbial Pathogenesis,* 136, 103716. https://doi.org/10.1016/j.micpath.2019.103716.
5. Cresti, L., Falciani, C., Cappello, G., Brunetti, J., Vailati, S., Melloni, E., Bracci, L. &Pini, A. (2022). Safety evaluations of a synthetic antimicrobial peptide administered intravenously in rats and dogs. *Science Reports,* 12(1), 19294. https://doi.org/10.1038/s41598-022-23841-2.
6. Eldin, A. B., Ezzat, M., Afifi, M., Sabry, O. &Caprioli, G. (2023). Herbal medicine: the magic way crouching microbial resistance. *Natural Product Research,* 1-10. https://doi.org/10.1080/14786419.2023.2172009.
7. Fagbemi, K.O., Aina, D.A. &Olajuyigbe, O.O. (2021). Soxhlet extraction versus hydrodistillation using the clevenger apparatus: A comparative study on the extraction of a volatile compound from *Tamarindus indica* seeds. *The Scientific World Journal,* 2021, 1-8. https://doi.org/10.1155/2021/5961586.
8. Firmino, D.F., Cavalcante, T.T., Gomes, G.A., Firmino, N., Rosa, L.D., de Carvalho, M. G. &Catunda Jr, F.E. (2018). Antibacterial and antibiofilm activities of *Cinnamomum sp.* essential oil and cinnamaldehyde: antimicrobial activities. *The Scientific World Journal*, 2018. https://doi.org/10.1155/2018/7405736.
9. Goyal, S., Gupta, N. & Chatterjee, S. (2016). Investigating therapeutic potential of *Trigonella* foenum-graecum L. as our defense mechanism against several human diseases. *Journal of Toxicology,* 2016. https://doi.org/10.1155/2016/1250387.
10. Hu, F., Tu, X.F., Thakur, K., Hu, F., Li, X.L., Zhang, Y.S., Zhang, J.G. &Wei, Z.J. (2019). Comparison of antifungal activity of essential oils from different plants against three fungi. *Food and Chemical Toxicology,* 134, 110821. https://doi.org/10.1016/j.fct.2019.110821.
11. Joshi, M.P., Chintu, C., Mpundu, M., Kibuule, D., Hazemba, O., Andualem, T., Embrey, M., Phulu, B. & Gerba, H. (2018). Multidisciplinary and multisectoral coalitions as catalysts for action against antimicrobial resistance: implementation experiences at national and regional levels. *Global Public Health,* 13(12), 1781-1795. https://doi.org/10.1080/17441692.2018.1449230.
12. Kalia, M., Yadav, V.K., Singh, P.K., Sharma, D., Pandey, H., Narvi, S.S. & Agarwal, V. (2015). Effect of cinnamon oil on quorum sensing-controlled virulence factors and biofilm formation in Pseudomonas aeruginosa. *PLoS one*, 10(8), e0135495. https://doi.org/10.1371/journal.pone.0135495.
13. Mittal, R.P., Rana, A. &Jaitak, V. (2019). Essential oils: an impending substitute of synthetic antimicrobial agents to overcome antimicrobial resistance. *Current Drug Targets,* 20(6), 605-624. https://doi.org/10.2174/1389450119666181031122917.
14. Mnif, S. & Aifa, S. (2015). Cumin (*Cuminum cyminum* L.) from traditional uses to potential biomedical applications. *Chemistry & Biodiversity*, 12(5), 733-742. https://doi.org/10.1002/cbdv.201400305.
15. Muhoza, B., Qi, B., Harindintwali, J.D., Koko, M.Y.F., Zhang, S. &Li, Y. (2023). Encapsulation of cinnamaldehyde: an insight on delivery systems and food applications. *Critical Reviews in Food Science and Nutrition,* 63 (15), 2521-2543. https://doi.org/10.1080/10408398.2021.1977236.
16. Ogbunugafor, H.A., Ugochukwu, C.G. &Kyrian-Ogbonna, A.E. (2017). The role of spices in nutrition and health: a review of three popular spices used in Southern Nigeria. *Food Quality and. Safety,* 1, 171-185. https://doi.org/10.1093/fqsafe/fyx020.
17. Paudel, S.K., Bhargava, K. &Kotturi, H. (2019). Antimicrobial activity of cinnamon oil nanoemulsion against *Listeria monocytogenes* and *Salmonella spp.* on melons. *Lwt*, 111, 682-687. https://doi.org/10.1016/j.lwt.2019.05.087.
18. Pingret, D., Fabiano-Tixier, A.S. &Chemat, F. (2014). An improved ultrasound Clevenger for extraction of essential oils. *Food Analytical Methods,* 7, 9-12. https://doi.org/10.1007/s12161-013-9581-0.

19. Prabuseenivasan, S., Jayakumar, M. &Ignacimuthu, S. (2006). *In vitro* antibacterial activity of some plant essential oils. *BMC Complementary & Alternative Medicines,* 6 (1), 1-8. https://doi.org/10.1186/1472-6882-6-39.
20. Prakash, V., Kumari, A., Kaur, H., Kumar, M., Gupta, S. &Bala, R. (2021). Chemical composition and antimicrobial activity of essential oil from aerial parts of *Origanum vulgare* L. from North-Western Himalayas. *Journal of Essential Oil Bearing Plants,* 24(2), 177-185. https://doi.org/10.1080/0972060X.2021.1919928.
21. Rao, A. S., Yadav, S. S., Sheoran, A., Singh, N., Nandal, A., Bhandoria, M. S., Ganaie, S. A. &Bansal, P. (2021). An ethnomedicinal survey of traditionally used medicinal plants from charkhidadri district, Haryana: An attempt towards documentation and preservation of ethnic knowledge. *Indian Journal of Traditional Knowledge*, 20, 436-450. http://op.niscpr.res.in/index.php/IJTK/article/view/31896/0.
22. Saki, M., Seyed-Mohammadi, S., Montazeri, E. A., Siahpoosh, A., Moosavian, M. &Latifi, S. M. (2020). In vitro antibacterial properties of *Cinnamomum zeylanicum* essential oil against clinical extensively drug-resistant bacteria. *European Journal of Integrative Medicine,* 37, 101146. https://doi.org/10.1016/j.eujim.2020.101146.
23. Singh, N. &Yadav, S.S. (2022a). A review on health benefits of phenolics derived from dietary spices. *Current Research in Food Science,* 5, 1508-1523. https://doi.org/10.1016/j.crfs.2022.09.009.
24. Singh, N. & Yadav, S.S. (2022b). Ethnomedicinal uses of Indian spices used for cancer treatment: A treatise on structure-activity relationship and signaling pathways. *Current Research in Food Science,* 5, 1845-181872. https://doi.org/10.1016/j.crfs.2022.10.005.
25. Singh, N. &Yadav, S. S. (2024). Antimicrobial and anticancer insights of cinnamaldehyde Schiff bases and metal complexes. *Inorganic Chemistry Communications*, 167, 112724. https://doi.org/10.1016/j.inoche.2024.112724.
26. Singh, N., Rao, A.S., Nandal, A., Kumar, S., Yadav, S.S., Ganaie, S.A. &Narasimhan, B., (2021a). Phytochemical and pharmacological review of *Cinnamomum verum* J. Presl-a versatile spice used in food and nutrition. *Food Chemistry,* 338, 127773. https://doi.org/10.1016/j.foodchem.2020.127773.
27. Singh, N., Yadav, S. S. &Narasihman, B. (2024). Antimicrobial and Antioxidant Assessment of *Trigonella* foenum-graecum. *Legume Research: An International Journal*, 7, 1113. https://doi.org/10.18805/LR-5348.
28. Singh, N., Yadav, S.S., Kumar, S. &Narashiman, B. (2021b). A review on traditional uses, phytochemistry, pharmacology, and clinical research of dietary spice *Cuminum cyminum* L. *Phytotherapy Research,* 35(9), 5007-5030. https://doi.org/10.1002/ptr.7133.
29. Singh, N., Yadav, S.S., Kumar, S. &Narashiman, B. (2022). Ethnopharmacological, phytochemical and clinical studies on Fenugreek (*Trigonella foenum-graecum* L.). *Food Bioscience,* 46, 101546.
30. Vázquez-Fresno, R., Rosana, A.R.R., Sajed, T., Onookome-Okome, T., Wishart, N.A. &Wishart, D.S. (2019). Herbs and spices-biomarkers of intake based on human intervention studies–a systematic review. *Genes & Nutrition,* 14, 18. https://doi.org/10.1186/s12263-019-0636-8.
31. Yang, K., Liu, A., Hu, A., Li, J., Zen, Z., Liu, Y., Tang, S. &Li, C. (2021). Preparation and characterization of cinnamon essential oil nanocapsules and comparison of volatile components and antibacterial ability of cinnamon essential oil before and after encapsulation. *Food Control*, 123, 107783. https://doi.org/10.1016/j.foodcont.2020.107783.
32. Yousefi, M., Rahimi-Nasrabadi, M., Pourmortazavi, S.M., Wysokowski, M., Jesionowski, T., Ehrlich, H. &Mirsadeghi, S. (2019). Supercritical fluid extraction of essential oils. *TrAC Trends in Analytical Chemistry,* 118, 182-193. https://doi.org/10.1016/j.trac.2019.05.038.
33. Yu, Z., Tang, J., Khare, T. & Kumar, V. (2020). The alarming antimicrobial resistance in ESKAPEE pathogens: Can essential oils come to the rescue? *Fitoterapia,* 140, 104433. https://doi.org/10.1016/j.fitote.2019.104433.