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ORIGINAL ARTICLE

Phytochemical and antimicrobial investigation of the leaves of five Egyptian mango cultivars and evaluation of their essential oils as preservatives materials

Salama A. Ouf[1] · Alaaeldin M. F. Galal[2] · Heba S. Ibrahim[3] · Amal Z. Hassan[2] · Maged K. G. Mekhael[2] · Khaled F. El-Yasergy[1] · Mohamed N. Abd El-Ghany[1] · Mohamed A. Rizk[1] · Atef G. Hanna[2]

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Abstract The sterols, hydrocarbons and fatty acids constituents of the leaves of five mango cultivars locally implanted in Egypt were identified. The effect of their essential oils (EOs) against food borne microorganisms was studied as preservative materials. The chemical constituents of the EOs isolated from mango leaves were identified by Gas Chromatography–Mass spectrometry (GC–MS) technique. Trans-caryophyllene, α–humulene and α–elemene were identified as terpene hydrocarbons, while 4-hydroxy-4-methyl-2-pentanone as oxygenated compounds were recorded in all tested cultivars with variable amounts. Results showed that *Staphylococcus aureus* and *Escherichia coli* were the most sensitive microorganisms tested for Alphonso EOs. On the other hand, *Salmonella typhimrium* was found to be less susceptible to the EOs of the studied cultivars. The EOs of different mango cultivars induced a steady decrease in the activity of amylase, protease and lipase at the minimum inhibitory concentration (MIC). The treatment of the tested bacteria with the EOs of mango cultivars caused a steady loss in enterotoxins even when applied at the sub-MIC. Bacteria-inoculated apple juice treated with minimum bactericidal concentration of Alphonso oil was free from the bacteria after 5 days of incubation at 25 °C. Eighteeen volatile compounds were found to reduce the activity of the amylase enzyme and the most active was cedrelanol (-7.6 kcal mol^{-1}) followed by alpha-eudesmol (-7.3 kcal mol^{-1}) and humulene oxide (-7 kcal mol^{-1}). The binding mode of both of cedrelanol and alpha-eudesmol with amylase enzyme was illustrated.

Keywords *Mangifera indica* · Essential oils · Antimicrobial activity · Food spoilage · Bacterial contamination · Food preservation

Introduction

Food spoilage and Food-borne diseases are still major problems in the world, even in well-developed countries. So, still a need for new methods of reducing or eliminating these difficulties, possibly in combination with existing methods and/or new one (s). A well-known novel way to reduce the proliferation of microorganisms is the use of plant-based essential oils (EOs) due to their less chance of being toxic (Fadel et al. 2019).

EOs are complex volatile compounds, synthesized naturally in various plant as secondary metabolites. Their importance arises from being bioactive as antifungal, antibacterial, antioxidant and anticarcinogenic properties (Tzortzakis 2007). The presence of different types of aldehydes, terpenes and phenolic compounds increases its activities against a diverse range of pathogens (Swamy

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✉ Salama A. Ouf
salama@sci.cu.edu.eg

✉ Atef G. Hanna
aghanna@hotmail.com

[1] Botany and Microbiology Department, Faculty of Science, Cairo University, Giza 12613, Egypt

[2] Chemistry of Natural Compounds Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, Giza 12622, Egypt

[3] Desert Research Centre, Medicinal and Aromatic Plants Department, Ecology and Dry Lands Agriculture Division, Cairo, Egypt

et al. 2016). Most of the EOs isolated from plants can be used as natural additives in many human foods. It has been proven that the inhibitory effect of EOs against a wide range of food-spoiling microbes, depends upon the concentration, method of testing and active constituents of EOs.

Mango (*Mangifera indica,* family Anacardiaceae) is native in the Indian subcontinent and introduced to other warm regions of the world including Egypt. The different parts of this plant were found to contain a series of polyphenols including: phenolic acids (gallic acid, 3,4-dihydroxy benzoic acid and benzoic acid), phenolic esters (gallic acid methyl ester, gallic acid propyl ester and benzoic acid propyl ester), flavan-3-ols (catechin, epicatechin and quercetin) and xanthones like mangiferin (Maurmanna et al. 2014). The peel extracts of cultivars of mango fruit revealed good antimicrobial activities against bacterial and fungal cultures and also possessed significant anti-inflammatory activity (Umamahesh et al. 2019). The hexane extract of mango fruits was found to contain alkanes, ketones, alcohols, esters and lactone in addition to sulfur and nitrogen as mineral components (Preethi and Saral 2014).

Myrcene, (Z)-ocimene and limonene were responsible for the green aroma, all were presented in the volatile oils of two Egyptian mango varieties (Alphonso and Baladi) (Engel and Tressl 1983). The EOs of the mango leaves were rich in sesquiterpenes (70.3%), while fruit peel oil consisted of very high amount of monoterpenes (83.2%) (Duresa 2017).

Previous research showed characteristic antimicrobial activity of several components of EOs including camphor, caryophyllene and humulene (Sabulal et al. 2006). Several studies revealed the possibility of utilizing active constituents of mango kernel, seed, fruits and bark as preservative materials against food-borne microorganisms. However, the phytochemical constituents of some local mango leaves cultivars were not previously studied in detail.

In the present article, a comparative study was undertaken to explore the phytochemical constituents of leaves from five Egyptian mango cultivars to comprehensively evaluate their antibacterial activities and the possibility of its using as food preservatives.

Materials and methods

Plant materials

Leaves of five different mango cultivars namely, Alphonso, Sidik, Ewase, Zebda and Fagri-kalan were collected from Faculty of Agriculture, Cairo University and were authenticated by Cairo University herbarium. Leaves were collected in the flowering stage during May and were frozen at 0 °C until used for isolation of EOs.

Phytochemical investigation of lipid constituents

Fresh leaves of mango (100 gm) were exhaustively extracted with petroleum ether (60–80 °C) at room temperature and the solvents were distilled off to give an oily extract. The extract was refluxed with 50 ml of 10% alcoholic KOH for 3 h and after cooling, it was further extracted with chloroform to give rise to the unsaponifiable matter (organic layer) and fatty acids (aqueous layer). The identification of hydrocarbon and sterol contents of the unsaponifiable matter was carried out by comparing the retention time of their peak with those of authentic samples (Hassan et al. 2017). The aqueous layer was acidified with 2 N HCl, followed by extraction with chloroform to afford the fatty acids fraction. The isolated fatty acids were methylated by refluxing with absolute methanol containing 5% H_2SO_4 for about one hour according to the method described by Hassan et al. (2017). Identification of the fatty acid methyl esters was achieved by comparing the retention time of their peaks with those of authentic by using GLC analysis.

Apparatus

The unsaponifiable matter and fatty acids were identified using GLC Hewlett Packard-HP 6890 series (United States), GC system, equipped with flame ionization detector. Nitrogen gas was used as carrier gas. The analysis of the unsaponifiable matter was carried out using capillary column (HP-1 methyl siloxane) and at oven temperature of 50 °C/11.7 min from 80 to 325 °C; injection and detector temperature was 300 °C. The operating conditions for fatty acid methyl ester analysis were capillary column polyethylene glycol (60 m × 320 mm), and the column temperature was 260 °C.

Isolation of the essential oil (EOs)

Fresh leaves (100 gm) of each of mango cultivar were exhaustively hydro-distilled using a Likens-Nickerson apparatus. The resulting oil was dried over anhydrous sodium sulfate and kept at 10 °C until analysis (Ahmed et al. 2015). The EO components were resolved and identified using GC–MS spectrometer.

Gas chromatography–mass spectrometry analysis

The analysis of the EOs was performed using gas chromatography–mass spectrometry (GC–MS) Finnegan mat

SSQ 7000. The model of GC is Trace GC 2000, produced by Thermo Finnegan Company, Italy, and injection volume was 1 µl, injection mode: split less, carrier gas was helium at the flow rate of 1.0 ml/min, main column (DB-5) and 5% phenyl methyl polysiloxan. Temperature program was 40 °C for the first 1 min, and then the temperature was increased to 160 °C at 3 °C/min for 40 min, which was then raised up to 250 °C by 2 °C/min for 45 min. MS model used was Finnegan SSQ 7000; mass range was 40–300, and scan time was 5 s. For GC–MS detection, an electron ionization system was used with ionization energy of 70 eV.

Test microorganisms

To evaluate the efficacy of the isolated EOs, five bacterial and 4 fungal species were used as test organisms in this research. The microorganisms have selected from the preserved and identified collection species of the first author (Ouf et al. 2016, 2018). These species were isolated from different food sources. The investigated bacterial species included two Gram + ve species namely; *Staphylococcus aureus*, *Bacillus cereus* and three Gram −ve ones; *Escherichia coli*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa*; while the test fungal species were *Aspergillus flavus*, *Cladosporum fulvum*, *Penicillium italicum*, *Rhizopus stolonifera* and *Ulocladium atrum*.

Determination of minimum inhibitory concentration (MIC) and minimum cidal concentration

MICs of EOs of each mango cultivar were determined by a two-fold serial dilution method (Chandrasekaran and Venkatesalu 2004). The tested EOs, were incorporated into broth medium to get a concentration of 4000 µg/ml (4 mg/ml). The oil was prepared in dimethyl sulfoxide, so that the separation of oil and water phases is not formed in the medium. A bifold series of dilution ranging from 2000 to 0.98 µg/ml was prepared in microtubes. Ten micro-liters of standardized suspension of each test bacterium (10^8 cfu/ml) or test fungus (10^5 cfu/ml) were transferred to each tube. The control tubes, containing only tested organism suspension, were incubated for 2 days at 37 °C in the case of bacteria and for 5 days at 27 °C for fungi. The lowest concentrations of the test samples, which did not show any growth of the tested organism after macroscopic evaluation, were determined as MICs. For determination of minimum cidal concentration, a loopful of broth from each test tube not showing growth, was inoculated into nutrient agar plate. Thereafter, equal volumes of sterile nutrient broth were added into the test tube cultures and incubated further for 24 h at 37 °C. Then, the tubes and agar plates were examined for growth or turbidity using unaided eye (CLSI 2012). A repeat of the dilution tube method with higher dilution instead of the double dilution with nutrient broth was done. These experiments were repeated three times. Minimum cidal concentrations (MBC for bacteria and MFC for fungi) are the least concentration of antimicrobial agent required to kill microorganisms.

Enzyme activity

Because of the EOs of the tested cultivars were significantly more effective towards the bacterial species compared to their efficiency against fungal species, therefore, the further work will be focused on studying the effect of the EOs on bacterial species. For all enzyme tests, the growth medium was inoculated with a suitable inoculum of each bacterium and supplemented with EOs to attain the MIC (MIC for each bacterium was prepared in dimethyl sulfoxide). Three replicates were used for each treatment.

Amylase activity

Amylase activity was measured by the dinitrosalicylic acid (DNS) method according to Khedr et al. (2017). The reaction mixture contained 1 ml of 1% soluble starch in an appropriate buffer and 1 ml of the fermentation broth, after removing the growth by centrifugation at 5000 rpm for 10 min. The mixture was incubated at 25 °C for 3 min. Two ml of DNS reagent were added and the mixture was boiled for 5 min. After cooling, the reaction mixture was diluted with distilled water and the absorbance was measured at 540 nm. One unit of amylase activity was defined as the amount of enzyme causing the release of 1 µmole of reducing sugars in one minute under the assay conditions using maltose standard curve. The enzymatic activity was expressed in U/ml, which is defined as µmoles maltose/ml of the culture filtrate per minute.

Protease activity

The reaction depends on that the enzyme hydrolyses peptide bonds of proteins liberating amino acids starting from the end. Precipitation with trichloroacetic acid (TCA) of all proteins after reaction is then done, and the amount of protein (as tyrosine) was measured according to Waterborg and Matthews (1984). Cultures were incubated at 30 °C for 48 h. The culture broth collected was filtered and termed cell-free filtrate.

One ml of soluble casein (1%) was added to 0.5 ml culture filtrate to 1.5 ml of 0.2 M Tris–HCl buffer, and then incubated at 37 °C for 30 min. One ml of TCA solution was added to stop the enzyme reaction and the mixture was cooled rapidly in ice. Then, the mixture was filtered through filter paper Whatman No. 3. The tyrosine

in the filtrate was estimated by the Folin reagents according to Lowry et al. (1951). The formed blue color of the reaction mixture measured spectrophotometrically at 750 nm. One unit of protease is defined as the amount of enzyme that releases 1 µg of tyrosine per ml per minute under the standard conditions.

Lipase activity

The activity of lipase was measured using an acid–base titrimetric assay, which is the most widely accepted method used to determine lipase activity (Jette and Ziomek 1994). The method depends on the measurement of the free fatty acid liberated from triacylglycerols substrates by enzymatic hydrolysis. The enzymatic reaction involves, in addition to substrate concentration, the size of the surface area of the oil–water interface in the assay system, where only sufficiently dispersed and stabilized oil/water emulsion are suitable for determination of lipase activity.

The lipase activity of the reaction mixture was assayed by measuring the free fatty acid released, by titration with 5 mM NaOH in the presence of 100 µl of phenolphthalein (1% w/v in ethanol) as indicator. Micromoles of oleic acid were obtained from the standard curve. One unit of lipase activity was defined as the amount of enzyme liberating one µ mol of oleic acid/min under standard assay condition.

Essential oils as preservative material for apple juice

Aliquots of 100 ml of commercial pasteurized apple juice were placed in sterile flasks. EO of Alphonso mango cultivar (being the most effective as antimicrobial) was added at its minimum bactericidal concentration for each bacterium to apple juice. 0.1 ml of the peptone-water culture of each test bacterium was inoculated into apple juice to obtain 4.0×10^6 cfu/ml. This was done for each bacterium separately. Throughout sample preparation and inoculation, rigorous measures were applied to prevent further microbial contamination. Inoculated samples were stored at $5 \pm 1^{\circ}C$ and 20 ± 1 °C. Counts of the test inoculated pathogens on tryptic soy agar (TSA) (Kang and Fung 2000) were determined at 1, 5 and 10 days of storage.

Molecular docking

The X-ray diffraction of the protein 1vem was downloaded from the protein data bank (https://www.rcsb.org/pdb/welcome.do). Its energy was minimized using the YASARA Energy Minimization Server (https://www.rcsb.org/pdb/welcome.do), all bound water, ligands, and cofactors were removed. The pdbqt file format of the protein and the 3D of the volatile compounds were created using MGL Tools 1.5.6 (www.mgltools.scripps.edu). Docking calculations were performed using Auto Dock Vena (Trott and Olson 2010) and PyRx 0.8 (www.mgltools. scripps.edu). The results were analyzed based on the binding of the ligand at the catalytic pocket using PyMol 1 (www.pymol.org).

Results and discussion

Chemical composition

The identification of the sterol content indicated that squalene was the major component in the leaves of Alphonso, Sidik, Zebda and Fagri-kalan cultivars (57.44, 29.54, 5.71 and 41.29%, respectively), while 7-dehydrocholesterol (46.51%) represents the major compound in the leaves of Ewase cultivar (Table 1). Lozano-Grande et al. (2018) reported that the presence of squalene in great amount leads to the stability of the oil. The presence of squalene as major component was, also remarked by Abdalla et al. (2007). Investigation of the hydrocarbon contents of different cultivars showed that docosane is the major hydrocarbon in the leaves of Alphonso, Sidik, and Fagri-kalan cultivars (7.83, 30.61 and 11.24%, respectively). The major hydrocarbon in Ewase cultivar was found to be heneicosane (8.32%). On the other hand, it was observed that the major hydrocarbon in Zebda cultivar was tetradecane (51.37%). The identification of heneicosan, tetradecan, hexadecane and pentadecane were reported earlier in alphonso mango fruit pulp (Idstein and Schreier 1985).

GLC analysis of the saponifiable matters of the five cultivars revealed the presence of saturated and unsaturated fatty acids with different concentrations. Behenic acid represents the higher content of the saturated fatty acids for Alphonso, Sidik, Ewase and Fagri-kalan cultivars (72.19, 50.02, 33.68 and 66.60%, respectively), while palmitic acid is the main identified fatty acid in Zebda cultivar. It was observed that linoleic acid was found in all tested cultivars except Zebda, while meristic acid was found in only three cultivar Ewase, Zebda and Fagri-kalan, also linolenic acid was present in only Alphonso, Sidik and Ewase cultivars (Table 1).

The present study of the lipids extracted from mango leaves showed that the fatty acid components approved the results reported by Gaydou and Bouchet (1984) stating that the fats content of mango kernel was oleic, palmitic, linoleic and stearic acid. On the other hand, the fatty acids composition of the seed kernel of Egyptian mango were found to be myristic, palmitic, stearic, oleic, linoleic and linolenic acid (Abdalla et al. (2007).

In our preliminary investigation, it was found that the total amount of EOs was affected by the time of collection

Table 1 Sterols, hydrocarbons and fatty acids of 5 mango cultivars according to the weight of the fresh cultivar leaves

Compound	Alphonso		Sidik		Ewase		Zebda		Fagri-kalan	
	RT*	%	RT	%	RT	%	RT	%	RT	%
Dodecane	–	–	2.51	0.20	3.45	0.33	2.72	11.67	3.20	
Tetradecane	6.09	2.83	5.57	0.06	–		5.61	51.37	6.61	0.35
Pentadecane	–	–	7.15	1.02	7.93	1.00	7.16	10.89	8.44	0.78
Hexadecane	8.79	6.38	8.55	0.14			8.5	5.45	9.94	0.78
Heptadecane	–	–	–	–	10.35	0.33	10.15	1.43		
Octadecane	–	–	11.55	3.68	12.61	1.99	12.69	0.39		
Nonadecane	–	–	12.97	1.64	13.98	3.32			13.65	2.26
Eicosane	–	–	14.29	5.73			14.23	2.72		
Heneicosane	15.96	7.09	16.79	6.95	16.09	8.32	16.75	1.82	15.84	2.53
Docosane	20.76	7.83	20.33	30.61	20.74	4.65	20.26	3.63	20.13	11.24
Squalene	23.64	57.44	23.63	29.54	23.60	33.55	23.53	5.71	24.74	41.29
7-dehydrocholesterol	25.85	2.12	26.95	20.25	26.99	46.51	26.95	4.92	26.73	14.63
Cholesterol	27.05	16.31								
Stigmasterol									33.84	24.83
Myristic acid					6.10	1.74	6.10	15.62	6.11	1.13
Palmitic acid	7.87	1.05	7.88		7.88	27.23	7.87	56.82		
Stearic acid	10.56	1.33	10.57	3.67	10.58	9.02	10.56	11.77	10.58	2.11
Oleic acid	11.10	1.45	11.00	14.68	11.00	13.65	10.99	7.14	11.00	1.04
Linoleic acid	11.77	1.23	11.75	7.25	11.75	4.39			11.75	2.70
Linolenic acid	12.65	22.75	12.63	12.48	12.60	10.29			12.65	26.43
Behenicacis	17.40	72.19	17.43	50.02	17.47	33.68	17.08	8.65	17.43	66.60

**RT* Retension time

and the internal time between collection and extraction. Accordingly, the plant collection was adjusted at 6 o'clock morning and followed directly by hydrodistillation using Likens-Nickerson apparatus.

Table 2 illustrates all the volatile compounds identified in the five mango cultivars. Analysis of the results showed that the major terpene hydrocarbon in Alphonso, Ewase and Zebda was trans-caryophyllene (18.88%, 38.80% and 12.71%, respectively) whereas, α –selinene (16.92%) and α-humulene (18.00%) were the major in Sidik and Fagri-kalan cultivars, respectively. Also, it was remarked that only three compounds of the terpene hydrocarbons were common in the tested mango cultivars, namely, α-elemet, trans-carophyllene and α-humulene. On the other hand, the major constituents of oxygenated compounds were Nerolidol (7.34%), (S)-α,α,4-trimethyl-3-Cyclohexene-1-methanol (10.10%), (S)-α,α,4-trimethyl-3-cyclohexene-1-methanol (1.63%), 4-hydroxy-4-methyl-2-pentanone (15.94%) and 2,5-Bis(1,1-dimethylpropyl)- 2,5-Cyclohexadiene-1,4-dione (7.48%) in the case of Alphonso, Sidik, Ewase, Zebda and Fagri-kalan cultivars, respectively.

Some of ketones such as cyclohexanone, camphor and caryophellene oxide were found in Alphonso, Ewase and Fagri-kalan cultivars only. With respect to the alcohol compounds, it was observed that 4-hydroxy-4-methyl-2-pentanone was present in all tested mango cultivars. On the other hand, linalool and (S)-α,α,4-trimethyl-3-cyclohexene-1-methanol could be detected in only three cultivars (Alphonso, Sidik and Ewase). Veridiflorol and [R-[R*, R*-(E)]]-3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol were detected only in Sidik and Ewase, respectively. As well, α-Eudesmol was detected in Sidik and Zebda respectively. Elemol was in Ewase and Zebda, methyl-camphenilol was in Alphonso and Sidik, 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol was in Alphonso and Ewase and Nerolidol was in Alphonso and Fagri-kalan. Acid ester compounds as dibutyl-1,2-benzenedicarboxylic acid ester was present in Alphonso, Sidik, Ewase cultivars. Eicosyl–oleic acid ester and octadecyl-9-octadecenoic acid (Z) -ester were common in Alphonso and Fagri-kalan, while octadecyl-9-octadecenoic acid was present in Ewase and Zebda cultivar. Moreover, ethyl ester octadecanoic acid, (E)-methyl-8-Octadecenoic acid ester, 1,2,3-propanetriyl-Docosanoic acid ester, propanoic acid, 2,2-dimethyl-,octyl ester were detected in only Zebda cultivar (Table 2).

The results reported in this article substantiate that reported by Claveiro et al. (1980), which isolated the EOs from mango leaves and show that they contain α- and *β*-pinene, Camphene, *β*-ocimene, *γ*-terpinene, α-terpinolene,

Table 2 Chemical composition of the five tested cultivars of mango leaves essential oil

Compounds	Alphonso		Sidik		Ewase		Zebda		Fagri-Kalan	
	RT*	(%)	RT	(%)	RT	(%)	RT	(%)	RT	(%)
4-hydroxy-4-methyl-2-Pentanone	6.93	4.51	6.90	0.44	6.99	1.36	7.03	15.94	7.03	4.46
Cyclohexanone	8.47	1.15			8.56	0.44	8.64	2.82	8.58	1.45
(-)-α-PINENE	9.68	0.61								
Linalool	15.16	0.87	15.16	1.23	15.20	0.15				
1-Terpineol	15.92	0.64								
3-methyl-camphenilol	16.82	1.17	16.84	0.42						
4-Terpineol	17.58	4.75	17.61	3.34	17.59	0.54				
(S)-α,α,4-trimethyl-3-Cyclohexene-1-methanol	18.08	18.4	18.15	10.10	18.05	1.63			18.06	4.21
(E)- 2-Decenal	20.05	0.53	19.95	0.50						
α-Damascenone	23.05	0.61								
α-Elemene	23.32	0.49	23.14	1.00	23.35	9.18	23.32	3.62	23.32	0.78
trans-Caryophyllene	24.16	18.88	24.21	8.06	24.22	38.80	24.11	12.71	24.14	15.70
2,5-bis(1,1-dimethylpropyl)- 2,5-Cyclohexadiene-1,4-dione	24.33	2.58	24.36	1.83					24.34	7.48
α-Humulene	25.07	16.45	25.10	8.48	25.12	25.98	25.03	11.07	25.05	18.00
Nerolidol	27.61	7.34							27.62	2.24
(Z)- benzoate 3-Hexen-1-ol	27.94	0.88								
(-)-Caryophyllene oxide	28.15	3.73			28.14	0.41			28.13	2.33
Humulene oxide	28.80	1.80			28.78	0.49				
Camphor	29.91	0.55	29.98	6.57	29.94	1.15			29.18	1.08
Heptadecane	30.74	0.48							30.75	0.77
6,10,14-trimethyl-2-Pentadecanone	33.75	1.26								
Nonadecane	34.95	0.64								
Dibutyl-1,2-benzenedicarboxylic acid ester	36.05	1.01	36.03	0.75	36.06	0.36				
Hexadecanoic acid	36.34	0.51								
Eicosane	36.90	0.64							36.91	0.66
Heneicosane	38.79	1.06							38.78	0.74
Docosane	40.58	1.25					42.24	1.26	40.58	0.80
Tricosane	42.31	2.12	42.30	0.88					42.31	2.17
Tetracosane	43.96	1.77	43.96	0.53					43.97	0.99
Pentacosane	45.60	2.32	45.59	0.70					45.60	1.57
eicosyl –oleic acid ester	46.93	0.73								
3-Ethoxy-2-butanone			6.25	0.51						
Cis-ocimene			13.10	0.50						
Borneol			17.42	0.71						
Para-cymene			18.88	0.39						
1,1,2,3,5,6-Hexamethyl-4-neopentyl-2–4-cyclohexadiene			22.32	0.74						
Germacrene A			23.40	10.96						
α-Gurjunene			23.88	12.21						
α-Guaiene			24.51	0.55	24.51	2.42	24.49	0.62		
γ-Selinene			25.48	3.09	25.48	3.28	25.46	4.72		
α-Selinene			26.00	16.92	25.91	5.17	25.88	4.33		
(-)-α-Panasinsen			22.66	1.08						
Palustrol			27.88	1.40						
Globulol			28.28	2.29						
Viridiflorol			28.49	1.40	29.18	0.54				
α-Eudesmol			29.17	0.47			29.90	2.06		
Octadecane			32.89	0.42					32.90	0.77

Table 2 continued

Compounds	Alphonso		Sidik		Ewase		Zebda		Fagri-Kalan	
	RT*	(%)	RT	(%)	RT	(%)	RT	(%)	RT	(%)
Ionol 2			39.11	0.42						
[R-[R,R-(E)]]-3,7,11,15-tetramethyl-2-Hexadecen-1-ol			39.32	1.12	39.49	0.21				
3-Methylene-2-pentanone					5.65	0.24				
3-methyl-6-(1-methyleth yl)-2-Cyclohexen-1-one					19.73	0.41				
α-Copaene					22.96	0.45			22.97	1.37
Isocaryophyllen					23.74	0.28				
cis-Guriune					25.64	0.68				
α-cadinene					26.58	1.24	26.57	0.64	26.57	5.02
Elemol					27.34	1.47	27.37	2.75		
Guaiol					28.48	0.49				
δ-Cadinol					29.54	0.70				
Phytol isomer					39.49	0.21				
octadec-9-enoic acid Octyl ester					41.10	0.2	41.13	8.47		
Octacosane					42.23	0.81				
4-methyl-3-Penten-2-one							5.78	5.23	5.67	0.69
4-methoxy-4-methyl-2-Pentanone							9.12	6.45		
3-methoxy-3-methyl-2-butanon							11.18	3.15		
2,2-Dimethy-propanoic acid octyl ester							14.29	3.25		
α-Terpinolene							14.64	3.02	14.65	12.09
ionone							18.20	0.54		
Germacrene B							24.34	1.03		
δ-Selinene							26.91	0.35		
1,2,2,6,8-Pentamethyl-7-oxabicyclo[4.3.1]dec-8-en-10-one							28.78	0.65		
2-Ethyl-hexanoic acid-2- ethyl-hexyl- ester							30.86	0.35		
Octadecanoic acid ethyl ester							36.92	0.57		
Heptacosane							40.60	0.44		
Nonacosane							43.19	1.89		
1-methyl-4-(1-methylethenyl)benzene									14.87	0.77
1,8-menthadien-4-ol									17.58	1.43
Para-cymen-8-ol									17.90	0.69
γ-Cadinene									25.50	1.17
Germacrene D									25.67	2.39
Eremophilene									25.91	0.80
Hexadecane									28.48	0.58
α-Cadinol									29.59	3.25
Trans-cadinol									29.85	3.55

linalool, *δ*- and *β*-elemene, *β*-caryophyllene, humulene, α-guaiene and *δ*-cadinene. On the other hand, Engel and Tressl (1983) reported that the aromatic principles of ripe mango were hydrocarbons, esters and alcohols. Also, Ragab and El-Nemr (1990) identified 64 volatile components in canned mango juice as 21 hydrocarbons, 25 alcohols and 2 aldehydes components and reported that *β*-caryophyllene and α -terpinolene were the major hydrocarbons. Idstein and Schreier (1985) reported that the major constituents of ripe Indian Alphonso mango fruit included (*E*)-ocimene. Engel and Tressl (1983) identified *β*-ionone in the volatile component of Alphonso and Baladi mango.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

In a preliminary investigation, the EOs of Ewase and Zebda cultivars were not significantly effective against the tested microorganisms even when applied at more than 2000 ppm, so the EOs of these cultivars were not included

in this and in the further experiments. The EOs of the other cultivars showed weak efficiency or ineffectiveness on fungi. On the other hand, the effect of EOs on bacteria appear significantly effective against the bacterial isolates depending on the mango cultivar and bacterial species. The most efficacious oil was that of Alphonso, while the most susceptible species was *E. coli* while *S. typhimurium* was relatively tolerant (Table 3).

The EO of Alphonso was the most effective oil and the MIC recoded 62.5 µg/ml for *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli* and measured 125 µg/ml for MBC. *Salmonella typhimurium* and *Pseudomonas aeruginosa* were less sensitive with 1000 and 500 µg/ml for MIC and > 2000 and 1000 µg/ml for MBC for the same species, respectively (Table 3). In most cases, it appears that the Gram −ve bacteria are less sensitive to EO than Gram + ve ones. However, this probably depends on the EO components of each mango cultivar rather than Gram characters. The EO of *Cordiaver benacea* contains mainly of α-pinene and trans-caryophyllene was active mainly against the tested Gram-positive bacteria and yeast (de Carvalho et al. 2004).

The bacteriostatic properties of the EOs of the tested cultivars are proved to contain several antimicrobial constituents including (S)-α,α,4-trimethyl-3-cyclohexene-1-methanol (18.40%), *trans*-caryophyllene (18.88%), and α-humulene (16.45%) in Alphonso cultivar; (*S*)-α,α,4-trimethyl-3-cyclohexene-1-methanol (10.10%), germacrene A (10.96%), α-gurjunene (12.21%), *trans*-caryophyllene (8.06%), α-humulene (8.48%), α-selinene (16.92%) and camphor (6.57%) in Sidik cultivar, and α-terpinolene (12.09%), *trans*-caryophyllene (15.70%), 2,5-bis(1,1-dimethylpropyl)-2,5-cyclohexadiene-1,4-dione (7.48%) and α-humulene (18.00%) in Fagri-Kalan cultivar (Table 2).

The variation in efficacy of the isolated EO components in this work in comparison with corresponding similar components achieved by other researchers may be due to many factors such as the geographical origin, genetic factors, the plant material and the season at which the plants were collected (Chang et al. 2008).

Purified compounds derived from EOs, such as carvacrol, eugenol, linalool, cinnamic aldehyde and thymol inhibit a wide variety of microorganisms (Ngome et al. 2018). Individual EO may contain complex mixtures of such compounds, however little is known about the effect of interactions between individual constituents on antimicrobial activity. Interactions between antimicrobials may lead to additive, synergistic or antagonistic effects (Davidson and Parish 1989).

The EOs rich in phenolic compounds such as carvacrol, oxygenated derivatives (thymol methyl ether) and its precursors *p*-cymene and c-terpinene were reported in all investigated cultivars and have described to possess high levels of antimicrobial activity (Memar et al. 2017). Moreover, studies reported that oxygenated terpenoids such as phenolic terpenes and alcoholic isolated and confirmed in this study have been approved to have marked antimicrobial activity than the other components due to their highly lipophilic nature and low molecular weight that induce it capable of disrupting the cell membrane, causing inhibiting the microbial and cell death (Kuspradini et al. 2018). Therefore, several in vitro tests indicate that terpenes or terpenoids show ineffective antimicrobial activity when used individually compared to in mixture with other components of EOs (Xue et al. 2013). Linalool and α-

Table 3 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), measured as µg/ml, of essential oils (EO) extracted from leaves of three cultivars of mango against the growth of food-borne microorganism

Species	Alphonso-EO		Sidik-EO		Fagri-Kalan-EO		Streptomycin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus aureus	62.5	125	125	250	250	500	3.9	15.6
Bacillus cereus	62.5	125	250	500	250	1000	3.9	15.6
Escherichia coli	62.5	125	62.5	125	125	250	3.9	15.6
Salmonella typhimurium	2000	> 2000	2000	> 2000	2000	> 2000	7.8	62.5
Pseudomonas aeruginosa	500	1000	1000	2000	2000	> 2000	7.8	31.3
Aspergillus flavus	1000	> 2000	> 2000	> 2000	> 2000	> 2000	62.5	125
Cladosporum fulvum	1000	2000	> 2000	> 2000	> 2000	> 2000	125	250
Penicillium italicum	2000	> 2000	> 2000	> 2000	> 2000	> 2000	62.5	250
Rhizopus stolonifer	> 2000	> 2000	> 2000	> 2000	> 2000	> 2000	62.5	125
Ulocladium atrum	> 2000	> 2000	> 2000	> 2000	> 2000	> 2000	125	250

Values are given as mean ± S.D of triplicate experiment. Streptomycin was used as reference and standard antibiotic

terpinol are known to have several biological activities such as antibacterial and antifungal (Park et al. 2012).

Enzyme activity

Table 4 shows that all the investigated bacteria are able to produce amylase, protease and lipase except *Salmonella typhimurium* and *Pseudomonas aeruginosa* which are incapable of produce amylase. *Staphylococcus aureus* was the most active species as enzyme producer under control condition. Application of the minimum inhibitory concentrations (MIC) of the oil to the growth medium induced a reduction in activities of the investigated enzymes of the tested bacterial species depending on mango cultivar and bacterial species. Alphonso oil was the most effective oil in the case of amylase inducing percentage reduction of 100, 97.2, 100 in the case of *Escherichia. coli, Bacillus cereus* and *Staphylococcus aureus*. The least effective oil was recorded in the case of protease recording reduction ranging from 10.3 to 17.8% for *Pseudomonas aeruginosa* and in the case of oil derived from Sidik and Fagri-Khalan, respectively.

For lipase, the reduction in enzyme activity was more practical against *S. aureus*, *B. cereus* and *E. coli* on application of oils from the three cultivars compared to the reduction recorded for *Salmonella typhimurium* and *S. aeruginosa*. The reduction reached more than 80% against *E. coli* in application of oils extracted from Alphonso and Sidik cultivars.

In addition to numerous metabolic products, the enzymes produced by microorganisms are considered the potential factors responsible for food deterioration and spoilage. The results (Table 4) reveals that the EOs of different mango cultivars achieved a significant decrease in the activity of amylase, protease and lipase at MIC.

There are few reports about the effects of plant derivatives on physiological attributes of the tested bacterial species including extracellular enzymes. The EOs inhibited α-amylase (EC50 = 88.9 ml/L) and α-glucosidase (EC50 = 71.94 ml/L) activities in a dose-dependent manner (Oboh et al. 2015). The extract of *Helichrysum italicum* reduced the activity of DNAse, coagulase, thermonuclease and lipase of *S. aureus* ATCC 6538 (Nostro et al. 2001).

Essential oils as preservative material for apple juice

In this experiment, pasteurized apple juice with the minimum bactericidal concentration of each strain was individually inoculated, each with a studied bacterium at approximately 10^4 cfu ml^{-1}, and stored at 5 °C and 25 °C. Counts on in Brain Heart Infusion broth (BHI, Difco Laboratories, Detroit, MI, USA) containing 10% glycerol were determined at 1, 5 and 10 days. The cultures were

Table 4 Effect of minimum inhibitory concentrations (MIC) of essential oils extracted from leaves of three mango cultivars on hydrolytic enzyme activity of the tested bacterial species calculated as Uml^{-1}. Percentage reduction is showed between bracts

Bacterial species	Amylase activity				Protease activity				Lipase activity			
	Control	Alphonso	Sidik	Fagri-Kalan	Control	Alphonso	Sidik	Fagri-Kalan	Control	Alphonso	Sidik	Fagri- Kalan
Staphylococcus aureus	920±12	0.0 (100)	43±7 (95.3)	61±6 (93.4)	85.5±5.2	32.3 ±4.0 (62.2)	41.1 ±5.1 (51.9)	55.0 ±4.1 (53.7)	220±31	54 ±4 (75.5)	82 ±7 (62.7)	99 ±8 (55.0)
Bacillus cereus	430±11	12±7 (97.2)	28±6 (93.5)	38±8 (91.2)	78.2±7.1	23.0 ±4.1 (70.4)	31.3±5.0 (80.0)	40.2 ±4.6 (48.6)	210±27	44 ±7 (79.1)	53 ±8 (74.8)	67 ±8 (68.1)
Escherichia coli	240±12	0 (100)	0 (100)	31±6 (87.1)	72.3 ±6.2	12.5 ±7.1 (82.7)	16.5 ±3.0 (77.2)	25.0 ±3.3 (65.4)	210±23	32 ±12 (84.8)	41±7 (80.9)	66 ±9 (68.6)
Salmonella typhimurium	0.0	0.0 (—)	0.0 (—)	0.0 (—)	77.0 ±4.0	51.0 ±6.2 (33.8)	55.9 ±6.2 (27.9)	61.3 ±7.3 (20.4)	235±31	101±11 (57.0)	121±6 (48.5)	139 ±13 (40.9)
Pseudomonas aeruginosa	0.0	0.0 (—)	0.0 (—)	0.0 (—)	64.0 ±7.0	53.1 ±7.3 (17.0)	57.4 ±7.1 (10.3)	52.6 ±8.0 (17.8)	211±29	120±11 (43.1)	139 ±14 (34.1)	153 ±7 (27.5)

inoculated during the exponential growth phase. Due to its high inhibitory level, Alphonso EO was used in this experiment.

At 5 °C, there was a significant suppression in counts of different bacterial species treated with the EO after one day (Table 5). After five and ten days, the bacterial counts of all species were suppressed in untreated and treated juice. However, the suppression was significantly higher in treated apple juice. With the exception of *Staphylococcus aureus*, no bacterial species was detected in juice treated with the EO after 10 days. Similar but faster trend of suppression in bacterial count of treated juice was obtained at 25 °C after 1, 5 and 10 days. The juice treated with oil was free from the bacteria after 5 days of incubation at 25 °C. Due to their antimicrobial, antioxidant, anticarcinogenic and antimutagenic properties, several EOs have been applied as promising natural preservatives. Thus, such plant-derived substances are considered to be safe alternatives to synthetic ones (Mittal et al. 2019). Moreover, EOs frequently revealed bacteriostatic effects comparable to or even stronger than some synthetic preservatives (Leja et al. 2019). Many natural preservatives have been widely used including citrus fruits by-products, green tea, bearberry, grape seed, rosemary and oregano (Pandey et al. 2017).

Virtual screening

Amylase is an enzyme found in bacteria, fungi and plants. The enzyme hydrolyzes starch, glycogen and related polysaccharides and oligosaccharides into glucose to supply the micro-organisms with energy. Consequently, inhibition of the enzyme can be led to the death of the microorganism by breaking down its food chain. The reduction of amylase activity illustrated in Table 4 encourages the authors to search about the possible role of volatile compounds in accomplishing this reduction.

In recent years, ligand docking in the binding site of a receptor protein is one of the most widely techniques used to predict the bioactivity of a set of compounds. The method depends on docking of energy minimized 3D ligand structure in the binding set of a definite 3D protein target followed by analysis of the results in comparison with the native ligand. Four crystal structures for bacterial beta-amylase enzyme in the protein data bank with IDs 1vem, 1ven, 1veo and 1vep were published. Analysis of the catalytic pocket of the given structures composes of eleven amino acids, Asp 49, His 89, Val 95, Glu 172, Arg 174, Thr178, Lys 287, Gly 290, Thr 330, Glu 367, Arg 397. The native ligand and its hydrogen bonds formed illustrated in Table 6. The virtual screening was carried out using Auto Dock Vina software for the 3D energy minimized structure of the identified volatile compounds isolated in this study.

Table 5 Count (cfu X 10^6/ml) of different bacterial strains inoculated individually, each in pasteurized apple juice supplemented with the minimum inhibitory concentration of essential oil of Alphonso mango cultivar, stored at refrigeration and room temperature[a]

Bacterial species	Initial count	5 °C						25 °C					
		Day 1		Day 5		Day 10		Day 1		Day 5		Day 10	
		With-out EO[b]	With EO	With-out EO	With EO	With-out EO	With EO	With-out EO	With EO	With-out EO	With EO	With-out EO	With EO
Staphylococcus aureus	4.28 ± 0.32	4.08 ± 0.31	3.82 ± 0.44	3.20 ± 0.42	2.21 ± 0.13	2.12 ± 0.21	1.12 ± 0.32	4.20 ± 0.65	3.30 ± 0.29	2.80 ± 0.27	ND	1.31 ± 0.07	ND
Bacillus cereus	3.95 ± 0.41	3.82 ± 0.41	3.22 ± 0.33	2.11 ± 0.11	1.88 ± 0.15	1.6 ± 0.11	ND	3.64 ± 0.45	2.95 ± 0.21	2.00 ± 0.18	ND	1.02 ± 0.09	ND
Escherichia coli	3.70 ± 0.22	3.54 ± 0.33	3.01 ± 0.32	1.62 ± 0.11	1.03 ± 0.28	0.85 ± 0.08	ND	3.32 ± 0.32	2.64 ± 0.32	0.88 ± 0.09	ND	ND	ND
Salmonella typhimrium	3.94 ± 0.11	3.65 ± 0.52	3.31 ± 0.32	1.87 ± 0.12	1.11 ± 0.27	0.91 ± 0.09	ND	3.02 ± 0.37	2.62 ± 0.26	1.03 ± 0.21	ND	ND	ND
Pseudomonas aeruginosa	3.97 ± 0.31	3.21 ± 0.24	2.22 ± 0.32	2.53 ± 0.26	1.74 ± 0.27	1.21 ± 0.32	ND	3.00 ± 0.51	1.83 ± 0.04	2.06 ± 0.21	ND	0.66 ± 0.11	ND

[a]Minimum inhibitory concentration (MICs) against the studied bacterial species were determined in Table 3

[b]EO = essential oil

Table 6 The volatile compounds that can be reduced the activity of the beta amylase enzyme according to the application of the virtual screening

Compounds	Binding Affinity (kcal mol^{-1})	RMSD*	Target amino acids
Native ligand	8.3	0	Asp 49, His 89, Val 95, Lys 287, Thr 330, Glu 367, Arg 397
1_Terpineol	−5.8	0	Glu 172, Gly 290
2_Decenal	−4.7	0	Gly 290, Thr 330
3_Ethoxy_butan_2_one	−4.7	0	Gly 290, Thr 330,
4_Hydroxy__4_methyl_2_pentanone	−4.6	0	Gly 290, Thr 330
4_Terpineol	−5.8	0	Glu 172, Thr178
Alpha_Eudesmol	−7.3	0	Gly 290, Thr 330
Benzoate_3_hexen_1_ol	−6.3	0	Gly 290, Thr 330
Damascenone	−6.5	0	Thr 178
Elemol	−6.5	0	Glu 172, Arg 174
Globulol	−6.4	0	Lys 287
Humulene_oxide	−7	0	Arg 397
Linalool	−5	0	Glu 172, Gly 290
Nerolidol	−6.3	0	Thr 330
Palustrol	−6.2	0	Arg 174, Tyr 178
Viridiflorol	−6.6	0	Thr 330
alpha_Cadinol	−6.7	0	Lys 287
cedrelanol	−7.6	0	Glu 172, Arg 174
p_Cymen_8_ol	−6	0	Gly 290, Thr 330

**RMSD* Root Mean Squared Deviations

The aim of the molecular docking calculation is to predict the possible compounds that can reduce the activity of the amylase enzyme. The docking into the catalytic pocket, forming hydrogen bonds with the previously mentioned amino acids and the binding affinity (kcal mol^{-1}) were considered the basic criteria for the activity of the volatile compounds. Table 6 illustrates that only 18 volatile compounds can reduce the activity of the beta amylase enzyme and the most active was cedrelanol (−7.6 kcal mol^{-1}) followed by alpha–eudesmol (−7.3 kcal mol^{-1}) and humulene oxide (−7 kcal mol^{-1}). In a trail to determine pharmacodynamic and pharmacokinetic properties of some biomolecules, Abhishek et al. (2019) docked 15 active compounds with Candidapepsin-1enzyme using the help of AutoDock 4.2.6 software. Ligustilide has the lowest free binding energy of − 5.75 kcal/mol against the fungal enzyme candidapepsin-1 with three hydrogen bond interactions at Ile 223, Tyr 225, and Thr 222 at the active site of the enzyme followed by cedrane with − 5.20 kcal/mol.

Conclusion

The present study was proceeded to investigate the phytochemical composition of the essential oils (EOs) of five Egyptian mango cultivars leaves, Alphonso, Sidik, Ewase, Zebda and Fagrikalan, to evaluate their antibacterial activities and the possibility of their using as food preservatives. The chemical constituents of the EOs were identified using GC–MS spectrometry showing the presence of biologically active chemical constituents such as trans-caryophyllene, α–humulene and α–elemene (terpene hydrocarbons), and 4-hydroxy-4-methyl-2-pentanone (oxygenated compounds) which were recorded in all tested cultivars with variable amounts. Results showed that *Staphylococcus aureus* and *Escherichia coli* were the most sensitive microorganisms tested for Alphonso EO. On the other hand, *Salmonella typhimrium* was found to be less susceptible to the EOs of the studied cultivars. The treatment of the tested bacteria with the EOs of mango cultivars caused a steady loss in enterotoxins even when applied at the sub-MIC (unpublished data). In summary, EOs isolated from the leaves of Alphonso cultivar of mango may display a more significant potential in food preservation against the toxigenic bacterial population contaminating plant juices.

Compliance with ethical standards

Conflicts of interest The authors declare no conflict of interest.

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