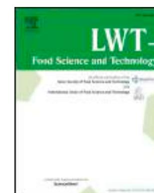
LWT - Food Science and Technology 104 (2019) 38–44

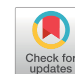
Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt



Chemical composition and biological activities of essential oil isolated by HS-SPME and UAHD from fruits of bergamot



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ARTICLE INFO

Keywords:
Ultrasound-assisted hydrodistillation (UAHD)
Headspace solid phase microextraction (HS-SPME)
Bergamot essential oil (BEO)
Antimicrobial activity
Antioxidant activity

ABSTRACT

Bergamot essential oil (BEO), which is extracted from bergamot fruit peel, has many health benefits such as improving blood circulation and anticancer activity. In this paper, volatile components were obtained by headspace solid phase microextraction (HS-SPME) and ultrasound-assisted hydrodistillation (UAHD) from the fruits of bergamot (*Citrus medica* L. var. *sarcodactylis* (Noot.) Swingle). The oil yield of BEO from UAHD extraction was 0.48%, which is 118% higher than that of hydrodistillation (HD). The chemical compositions of the volatile components were analyzed using GC-MS. A total of 46 volatile compounds belonging to distinct chemical families were identified. The major components were identified as D-limonene (60.44%), γ-terpinene (20.28%). The biological activities, including antimicrobial activities and antioxidant activities of BEO were detected. The BEO exhibited activities against bacteria and fungi with minimal inhibitory concentration (MIC) values in the range of 0.78–3.13 μL/mL and minimum bactericidal or fungicidal concentration (MBC/MFC) values in the range of 1.56–6.25 μL/mL. BEO showed strong activity against *E. coli* (MIC = 0.78 μL/mL, MBC = 1.56 μL/mL). BEO also showed substantial antioxidant activity based on ferric reducing antioxidant power (FRAP) assay for reducing power, 2,2′-azino-bis(3-ethyl benzothiazoline-6-sulfonic acid) (ABTS) assay for radical scavenging activity, and superoxide anion radical scavenging activity.

1. Introduction

The concept of functional foods that combine nutritional and medicinal benefits has been developed in recent years. Many natural compounds extracted from plants exhibit biological activities (Contreras-Moreno et al., 2016; Dong et al., 2015; Dra et al., 2017). Essential oils have been used as additives to food, beverages, cosmetics, chemical cleaners and medicines. They exhibit high levels of antimicrobial (antiseptic), antioxidant and anticancer activities (Basappa et al., 2015; Barros, Morais, Ícaro Gusmão Pinto Vieira, Craveiro, & Sousa, 2015; Hossain & Shah, 2015). The antioxidant and antimicrobial activities of essential oils have become hot research topics. Many essential oils qualify as natural antioxidant and antimicrobial agents and the potential impact on the food preservation based on these properties has received increasing attention in recent years (Ghabraie, Vu, Tata, Salmieri, & Lacroix, 2016; Hafsa et al., 2016; Kazemi, 2014).

Bergamot has been used as a medicinal plant because of its stomachic, anti-fungal and bacteriostatic properties of its fruit (Jin et al., 2016; Scuteri et al., 2018). Bergamot essential oil (BEO) is an important widely used product in many flavors and perfumes (Verma et al., 2016). BEO is a well-characterized plant extract in great demand by cosmetic and perfumery industries. BEO has been used in drug industry because of its antibacterial and antioxidant properties and also in the food products as a flavoring in teas, candies, ice creams, and soft drinks (Jiang, Luo, & Ying, 2015; Watanabe et al., 2015). Recently, BEO used for food preservation has received increased attention within the food industry, because it can be used as a natural alternative to chemical preservatives in line with changes in legislation and consumer trends (Avila-Sosa, Navarro-Cruz, Sosa-Morales, López-Malo, & Palou, 2016; Ekpenyong & Akpan, 2015). Nevertheless, few published papers have

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https://doi.org/10.1016/j.lwt.2019.01.020
Received 22 May 2018; Received in revised form 26 November 2018; Accepted 15 January 2019
Available online 17 January 2019
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reported on the antioxidant properties of BEO. There are also few reports comparing the antimicrobial activities of major chemical constituents of BEO.

There are various techniques available for extracting volatile components from plant materials. These include hydrodistillation (HD), supercritical fluid extraction (SFE) and solid-phase microextraction (SPME). HD is a conventional method used to extract essential oils from aromatic plants. The HD process can be easily scaled up in industry and results in little chemical pollution, but it has disadvantages including: i) heat-sensitive compounds can be destroyed by high temperature; and ii) low extraction yields of essential oils. Based on these limitations, it is necessary to develop an alternative extraction process that does not modify the composition of raw material and uses less solvents and energy. Ultrasound has many effects which includes assistance for the extraction, it depends on the power and frequency etc. High intensity of ultrasound could degrade some molecules or promote some reactions thus the final products might be different (Ren et al., 2019; Wu, Zhang, Jia, Kuang, & Yang, 2018). And they have some other effects including enhance the dispersion or separation (Zhang, Zhao, Lai, Chen, & Yang, 2018; Zhao, Zhang, & Yang, 2017). Ultrasound-assisted hydrodistillation (UAHD) is a rapid, efficient and low cost extraction method, which has been widely applied in the food, chemical and pharmaceutical industries recently. Therefore, the use of UAHD to extract the essential oil from Bergamot is promising. Headspace solid phase microextraction (HS-SPME) has been widely used for isolation and determination of volatiles from fruits, wines and spices since its invention in 1989 (Ye et al., 2017). HS-SPME is a simple, sensitive and fast method to analyze the natural fragrant components of plants. One of the trends of SPME is the combination with nanotechnology to enhance the specificity of the extraction efficiency, which is important for enhance the effectiveness of the analysis (Yu, Ang, Yang, Zheng, & Zhang, 2017; Yu et al., 2019; Yu, Li, Ng, Yang, & Wang, 2018; Yu & Yang, 2017).

The aim of the current work was the extraction of the BEO by UAHD and volatile components from bergamot by HS-SPME, followed by their GC-MS analysis. The volatile composition of BEO and bergamot were compared, and the antimicrobial and antioxidant activities of BEO were evaluated.

2. Materials and methods

2.1. Materials

Bergamot (*Citrus medica* L. var. *sarcodactylis* (Noot.) Swingle) was purchased from Jinhua market (Zhejiang Province, China) and identified by Prof. Lixing Hong, Zhejiang Academy of Forestry. 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), L-ascorbic acid and all the pure essential oil components were purchased from Sigma–Aldrich (St. Louis, MO, USA). Tetracycline hydrochloride and amphotericin B were purchased from Aladdin Bio-Chem Technology (Shanghai, China). All the chemicals and solvents used in this study were of analytical grade.

2.2. Essential oil extraction by UAHD

Dried bergamot (200 g) was cut into small pieces, and then placed in a 5000 mL round-bottomed flask with 2000 mL distilled water (Ai, Mouhouche, Hazzit, & Ferradji, 2016). The round-bottomed flask was placed in an ultrasonic bath (KQ-300GVDV, Kunshan Hechuang Ultrasonic Instrument Co., Ltd. China). The extraction times, using 180 W of ultrasonic power, included 20, 30, 40, 50, 60 and 70 min. After ultrasonic treatment, the flask was put onto a distillation apparatus for 3 h to obtain the essential oil using a Dean-Stark apparatus. Finally, the essential oil was separated from water, dried using anhydrous sodium sulfate, and stored in brown bottle at 4 °C for further analysis.

2.3. Volatile compounds by HS-SPME

In HS-SPME process (Gonçalves, Figueira, Rodrigues, & Câmara, 2015), divinylbenzene-carboxen-polydimethylsiloxane fibers (DVB-CAR-PDMS, 30 μm) were used to obtain the volatile constituents in bergamot. Dried bergamot powder (1 g) was put into a 20 mL headspace vial. The headspace vial was incubated in 40 °C water bath for 15 min, then the fiber was exposed to the sample headspace for 40 min and inserted directly into the gas chromatography (GC) injector port at 250 °C for 5 min.

2.4. Gas chromatography-mass spectrometry (GC–MS) analysis

The volatile components were analyzed using a 7890A Network GC System (Agilent Technologies Inc., Palo Alto, CA), equipped with a HP-5MS capillary column and a 5975C Network inert MSD mass spectrometer, operated in electron impact ionization mode at 70 eV and the mass range scanned was 50–500 amu in full-scan acquisition mode. The injection and ion source temperatures were set at 250 and 150 °C, respectively. The gradient of temperature was applied (50 °C for 1 min, then raised up by 5 °C/min to 130 °C, held at 130 °C for 0.5 min, increased by 15 °C/min to 250 °C, then held at 250 °C for 10 min). The flow rate of carrier gas (helium) was 1 mL/min. The mass spectra were processed using mass spectra search (version 2.0, National Institute of Standards and Technology). Retention indices were calculated using a standard mixture of homologue hydrocarbons (C_7–C_{26}).

2.5. Antimicrobial activity

2.5.1. Microorganisms

The antimicrobial activity (Ahmad, Mohammad, Amin, & Mojtaba, 2018; Contreras-Moreno et al., 2016) of BEO and its key components were tested against three Gram-positive bacterial strains (*Bacillus subtilis, Micrococcus luteus* and *Staphylococcus aureus*), one Gram-negative bacterial strain (*Escherichia coli*) and a single yeast strain (*Saccharomyces cerevisiae*). The microorganisms were obtained from Microbiology Laboratory, Zhejiang University of Technology, China. They were maintained at 4 °C on slants of Nutrient Agar (0.5% peptone, 0.3% beef extract, 1.5% agar, 0.5% sodium chloride and distilled water) for bacteria, and potato dextrose agar medium (0.4% potato, 2% dextrose, 1.5% agar powder and distilled water) for the yeast. Active cultures were prepared by transferring a ring loop of cells from the agar slant to a test tube containing 5 mL of nutrient broth for bacteria and yeast. The bacterial and yeast cultures were then incubated overnight at 37 °C (6–10 h) and 30 °C (12–16 h) respectively.

2.5.2. Determinations of MIC and MBC/MFC

MIC and MBC/MFC were determined by double broth dilution method (Kazemi, 2014; Upadhyay, 2015; Verma et al., 2016). All tests were performed on nutrient agar medium (for bacteria) and potato dextrose agar medium (for yeast). The BEO and its key components were dissolved in 1% dimethylsulfoxide (DMSO) and then diluted to a concentration of 50 μL/mL. A serial dilutions of BEO and its key components were performed in 96 well microtiter plates to obtain the desired concentrations ranging from 0.02 to 50 μL/mL The final microbial populations (100 μL in each well) was adjusted to 2.0×10^6 CFU/mL for bacteria and 2.0×10^5 CFU/mL spore for yeast. The microplates with bacteria were incubated at 37 °C for 24 h and with yeast were incubated at 26 °C for 48 h. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of BEO that inhibited the visible growth of a microorganism. The microbial growth was determined by a universal microplate reader (DG5033A) at 600 nm (Nanjing Huadong Electronics Group Medical Equipment Co, Ltd. China).

To determine minimum bactericidal concentration (MBC), 0.1 mL of the culture in each well at MIC (with no visible growth) was spread on

nutrient agar medium for 24 h at 37 °C for bacteria or on potato dextrose agar medium for 48 h at 26 °C for the yeast. The MBC/MFC was defined as the lowest concentration of the essential oil at which 99.5% of inoculated microorganisms were killed (Nikolić et al., 2013). All determinations were performed in triplicate and two growth controls consisting of medium with 1.0% (v/v) DMSO were included. Tetracycline hydrochloride and amphotericin B served as positive controls.

2.6. Antioxidant activity

2.6.1. Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) of sample was determined according to the reported method with slight modifications (Abdelwahab et al., 2017; Benzie & Strain, 1996). To prepare the FRAP reagent, 0.1 M acetate buffer (pH 3.6), 10 mM tripyridyltriazine (TPTZ), and 20 mM ferric chloride (10:1:1, v/v/v) were combined. The diluted sample (150 μL) was added to 2850 μL of the prepared FRAP working solution and vortexed thoroughly. The absorbance was then measured at 593 nm after the mixture was incubated for 30 min in dark at room temperature. Blank sample was prepared by omitting $FeCl_3$ from FRAP solution using methanol instead. L-ascorbic acid served as positive control.

2.6.2. ABTS radical scavenging activity

The ABTS free-radical-scavenging activity of the extracts was assayed according to a previous protocol (Urbizu-Gonzlez & Castillo-Ruiz, 2017) with some modifications. ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS stock solution (7 μM in water) with 2.45 μM potassium persulfate (final concentration) and kept in the dark at room temperature for 12–16 h. The ABTS storage solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm at the time of analysis; 1 mL-diluted sample was mixed with the $ABTS^{\cdot+}$ solution (2 mL) and votexed intensely. The reaction mixture was kept at room temperature for 30 min in dark, and the absorbance was measured at 734 nm. L-ascorbic acid served as positive control. The $ABTS^{\cdot+}$ radical scavenging effect was calculated as follows:

$$\text{ABTS}^{\cdot+}\text{scavenging effect (\%)} = \left[\frac{A_0 - A_1}{A_0} \times 100\%\right]$$

where A_0 is the absorbance of blank sample (containing all reagents except the test compound) and A_1 is the absorbance of the sample. L-ascorbic acid served as positive control.

2.6.3. Superoxide radical ($O2^-$) scavenging activity

The influence of essential oil from fruits of bergamot on the generation of superoxide anion was measured according to the method described in previous work (Senthilkumar & Venkatesalu, 2013; Zhang, Li, Xing, Yang, & Sun, 2014) with some modifications. Superoxide anion was generated in a non-enzymic system and determined by a spectrophotometric measurement for reduction of nitro blue tetrazolium. The reaction mixture, which contained 1 mL extract in distilled water, 1 mL phenazine methosulfonate (PMS) (60 μM) in sodium phosphate buffer (0.1 M, pH 7.4), 1 mL NADH (468 μM) in sodium phosphate buffer and 1 mL nitroblue tetrazolium chloride (NBT) (150 μM) in sodium phosphate buffer, was incubated at an ambient temperature for 5 min, and the color was detected at 560 nm against blank samples. All analyses were run in triplicate and mean values were calculated. The superoxide anion scavenging activity was calculated by using the equation:

$$\text{Superoxideanionscavengingeffect (\%)} = \left[\frac{A_0 - A_1}{A_0} \times 100\%\right]$$

where A_0 is the absorbance of blank sample (containing all reagents except the test compound) and A_1 is the absorbance of the sample.

2.7. Statistical analysis

All analyses were carried out in triplicate. The antimicrobial and antioxidant results were expressed as mean values ± standard deviation (SD). Differences among samples were tested with analysis of variance (ANOVA) followed by multiple-comparison test (Tukey HSD). In necessity, data were transformed to satisfy normal distribution and homoscedasticity requirements. If transformed data could not meet these assumptions, differences were analyzed with nonparametric analysis of variance (Kruskall-Wallis) followed by nonparametric multiple-comparison test (Mann-Whitney). All statistical analyses were tested at the basis of mean values to determine the significance at $p < 0.05$ with the software of SPSS 17.0.

3. Results and discussion

3.1. Essential oil extraction

Two methods (UAHD and HD) for BEO extraction were applied and compared. The elevation of ultrasound-assisted extraction time from 20 to 60 min resulted in an increased in BEO extraction yield. The optimized ultrasonic extraction time 60 min was used for the UAHD of oil from bergamot (Fig. 1). The oil yield of BEO from UAHD extraction was 0.48%, which is 118% higher than that of HD (0.22% only). The ultrasound was applied in UAHD extraction as a disruptor of the BEO glands in order to release the essential oil.

The composition of essential oil obtained by UAHD was analyzed by GC-MS (Fig. 2 and Table 1). The results show nineteen components, representing 96.66% of the total volatiles, were identified based on the NIST mass library and literature. According to our results, the major components of the identified BEO were D-limonene (60.44%), γ-terpinene (20.28%), α-terpineol (2.33%), β-bisabolene (2.03%), *m*-cymene (1.88%), *p*-menth-1-en-4-ol acetate (1.76%), β-caryophyllene (1.57%), α-pinene (1.27%), β-terpinene (1.23%) and δ-elemene (1.06%). Monoterpenes were the main substances, and the relative contents of D-limonene and γ-terpinene were much higher than in most other ingredients. Lin et al. used steam distillation to extract BEO and the main chemical components were olefins, alcohols and aldehydes by GC-MS analysis and its percentage was 39.75%, 24.73%, and 26.38%, respectively (Lin et al., 2015). Yang et al. extracted BEO by low temperature continuous phase change extraction technique, and obtained the main chemical components of acid, olefin and ester. The relative percentages were 44.3%, 26.67% and 7.89%, respectively (Yang et al., 2015).

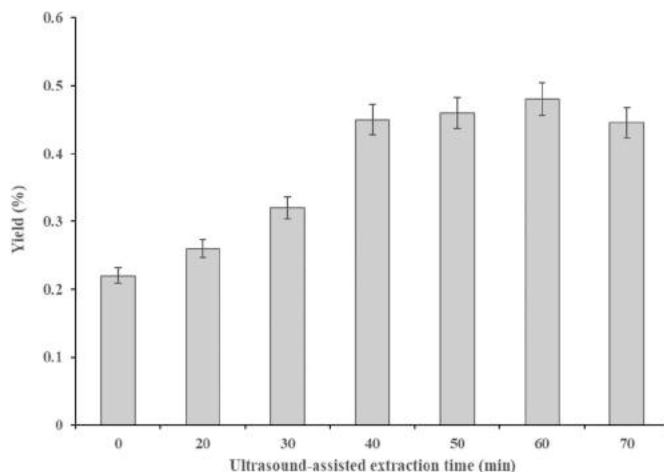

Fig. 1. Effect of UAE time on the oil extraction yield of bergamot. Extraction at UAE time from 20 to 70 min, power 180W.

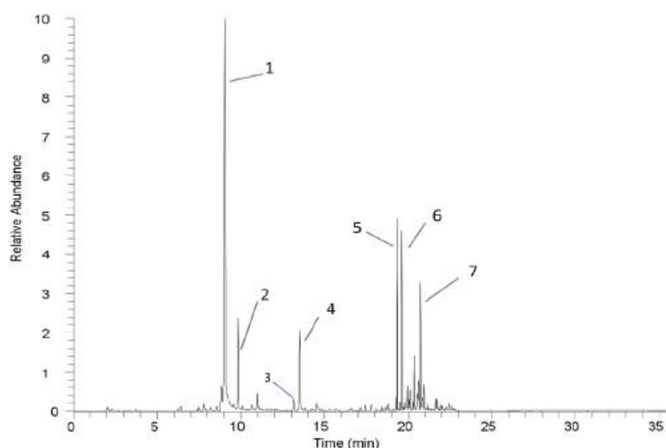

Fig. 2. The total ion chromatograms of aroma components of bergamot by GC-MS. (1) D-limonene; (2) γ-terpinene; (3) terpinen-4-ol; (4) α-terpineol; (5) β-caryophyllene; (6) α-bergamotene; (7) L-bisabolene.

3.2. Analysis of volatile compounds from HS-SPME extraction

Forty-six volatile compounds were identified from the samples prepared by HS-SPME, representing 94.38% of the total volatiles mass. The major components were identified as D-limonene (34.81%), β-caryophyllene (9.91%), α-bergamotene (8.33%), γ-terpinene (5.54%), α-terpineol (5.53%), β-bisabolene (5.51%), β-cubebene (2.82%), elixene (2.27%), *m*-cymene (2.15%), linalool (1.61%), α-caryophyllene (1.38%) and δ-cadinene (1.37%). Monoterpenes and its oxides were the main substances. The relative contents of D-limonene and γ-terpinene in HS-SPME volatile products were lower than that in the product extracted using UAHD, while the contents of α-bergamotene and β-bisabolene were relatively higher.

Much more compounds were identified (Table 1) in the BEO by HS-SPME than in the BEO by UAHD (46 compounds *vs*. 19 compounds). The difference may due to the loss of some unstable and oxidative components during the process of UAHD (Omar, Yusoff, & Ibrahim, 2017). Our volatile composition of BEO was similar to the reported data showing the most abundant compound was limonene, followed by linalyl acetate, linalool, γ-terpinene and β-pinene (Lin et al., 2015).

3.3. Antimicrobial activity

The antimicrobial activities of the BEO were evaluated using a series of important bacterial strains (including Gram-positive and Gram-negative strains) and yeast strain. The antimicrobial activities were compared with commercial antibiotics (tetracycline hydrochloride and amphotericin B). Antimicrobial activities of BEO (as MIC and MBC/MFC values) are summarized in Table 2. The results show that the MIC of BEO at 0.78 μL/mL was found to be the most effective against *E. co*li. In regards to the Gram-positive bacteria, a MIC of BEO at 1.56 μL/mL was found to be more effective against *S. aureus*. The fungal strain viz., yeast was also susceptible to the BEO showing a MIC = 1.56 μL/mL. The bacteria viz., *E. coli*, *S. aureus* and yeast were found to be more susceptible to the BEO with a MBC value of 1.56 μL/mL, while *M. luteus* was found less susceptible to the BEO (MBC > 6.25 μL/mL). Apparently, the inhibitory effect of BEO on Gram-negative strains is stronger than that on Gram-positive strains.

Next, the antimicrobial activities tested two major constituents, D-limonene and γ-terpinene from BEO using the same experimental conditions and strains. The results are also summarized in Table 2. According to the MIC, MBC and MFC values, the major constituents of the BEO displayed lower antimicrobial activity than BEO. It is apparent that D-Limonene shows significant activity against *yeast* (MIC = 6.25 μL/mL, MBC = 6.25 μL/mL). The antifungal activity of BEO may be largely due to D-Limonene, accounts for 60.44% of the essential oil. In addition, γ-terpinene has similar antimicrobial activity on the Gram-positive, Gram-negative and yeast strain. A previous study indicated that D-Limonene has strong inhibition effects on the Gram-positive and fungi strains (Semeniuc, Pop, & Rotar, 2016). Major compounds might be the basis for the antimicrobial activity exhibited. Possible synergistic effect of the major compounds in the essential oil can also been taken into consideration (Kazemi, M. 2014). According to Jiang et al. (2015) reports, the principal mechanisms of action of BEO major compounds (D-limonene and γ-terpinene) are against the cytoplasmic membranes of microorganisms, causing a loss of membrane integrity; the inhibition of respiratory enzymes; and dissipation of the proton-motive force (Zhang, Vriesekoop, Yuan, & Liang, 2014). It is suggested that BEO have good antimicrobial activity. For the application, the delivery of this essential oil to make it effective and have a relative long time effect are critical as the volatile nature of essential oil. Sow, Tirtawinata, Yang, Shao, and Wang (2017) combined carvacrol with nanoemulsion to explore the antimicrobial activity, the results showed that carvacrol nanoemulsion can inhibit the bacteria, yeast *in vitro* and native microflora on shredded cabbaged, and has good antibacterial activity (Sow et al., 2017).

3.4. Antioxidant activity

Owing to the complexity of phytochemicals in essential oils, no single assay will accurately reflect all antioxidants in such complex system. For this reason, the antioxidant activity of BEO was demonstrated by three spectrophotometric methods, FRAP assay for reducing power, ABTS assay for radical scavenging activity and superoxide anion radical ($O2^-$) scavenging activity.

3.4.1. Ferric reducing antioxidant power (FRAP)

The principle of the analytical method is that the antioxidant substance reduces Fe^{3+} to Fe^{2+}, and Fe^{2+} combines with TPTZ to form a blue complex, which has maximum light absorption at 593 nm, and the absorbance value is larger. It shows that the stronger the reducing ability of the antioxidant, the higher the antioxidant activity.

BEO shows a linear relationship for FRAP at concentrations from 0.1 to 10 mg/mL (Fig. 3A). The reducing power increased with the increased absorbance from 0.01 to 1.30. The results show that the scavenging effect of BEO is concentration dependent. Abdelwahab et al. (2017) performed an antioxidant assay on *C. altissimum* and the FRAP value of the sample was 345.2 ± 14.8 (μMFe(II)/g dry mass), indicating that it had a certain reducing ability and the FRAP value was lower than ascorbic acid (Abdelwahab et al., 2017).

3.4.2. ABTS radical scavenging activity

As shown in Fig. 3B, the clearance rate of ABTS-free radicals by BEO increases with increasing concentration. At concentrations of 0.1 mg/mL, 0.5 mg/mL, 1 mg/mL, 5 mg/mL and 10 mg/mL, the clearance rates of BEO to ABTS-free radicals were 2.06%, 12.81%, 29.29%, 78.26%, and 86.5%, respectively. It can be seen that the BEO has a scavenging effect on ABTS-free radicals and has antioxidant capacity. Jiang et al. (2015) used several different essential oils to fumigate fresh mushrooms, the results showed that the antioxidant activities of the mushrooms fumigated were significantly increased and extended the shelf life of shiitake mushrooms (Jiang et al., 2015).

3.4.3. Superoxide radical-scavenging activity

BEO showed obvious $O2^-$ scavenging activity that is dependent on the concentration of BEO (Fig. 3 C). The clearance rate of BEO to $O2^-$ increases with increasing concentration. At concentrations of 0.1 mg/mL, 0.5 mg/mL, 1 mg/mL, 5 mg/mL, and 10 mg/mL, the clearance rates of bergamot essential oils for $O2^-$ were 0.67%, 2.33%, 10.32%, 38.27%, and 81.36%, respectively. At a concentration of 10 mg/mL, the BEO removal rate is slightly lower than the vitamin C clearance rate, so the bergamot essential oil has a good ability to remove $O2^-$ at a certain

Table 1
Volatile compounds identified by GC-MS in the fruit of bergamot.

No	Compounds	molecular formula	RI[d]	Content(%)		RT[c]	
				HS-SPME[a]	UAHD[b]	HS-SPME	UAHD
1	3-methylene-6-(1-methylethyl)-Cyclohexene	$C_{10}H_{16}$	918	0.19	–	6.23	–
2	α-Pinene	$C_{10}H_{16}$	924	0.31	1.27	6.4	6.747
3	β-Terpinene	$C_{10}H_{16}$	962	0.14	1.23	7.37	7.854
4	Sabenene	$C_{10}H_{16}$	965	0.28	–	7.47	–
5	α-Myrcene	$C_{10}H_{16}$	978	0.62	–	7.81	–
6	α-Phellandrene	$C_{10}H_{16}$	992	0.24	–	8.18	–
7	Terpinolene	$C_{10}H_{16}$	1005	0.40	–	8.52	–
8	m-Cymene	$C_{10}H_{14}$	1016	2.15	1.88	8.84	9.138
9	D-Limonene	$C_{10}H_{16}$	1031	34.81	60.44	9.05	9.264
10	γ-Terpinene	$C_{10}H_{16}$	1051	5.54	20.28	9.85	10.096
11	δ-Elemene	$C_{15}H_{24}$		–	1.06	–	10.935
12	Linalool	$C_{10}H_{18}O$	1090	1.61	–	10.98	–
13	Terpinen-4-ol	$C_{10}H_{18}O$	1164	0.93	1.76	13.13	13.467
14	α-Terpineol	$C_{10}H_{18}O$	1178	5.53	2.33	13.53	13.835
15	β-Nerol	C10H18O	1213	0.94	–	14.54	–
16	Geraniol	$C_{10}H_{18}O$	1238	0.33	–	15.25	–
17	ascaridole epoxide	$C_{10}H_{16}O_3$	1285	0.33	–	16.58	–
18	Elixene	$C_{15}H_{24}$	1300	0.36	–	17.44	–
19	a-Cubebene	$C_{15}H_{24}$	1316	0.49	–	17.78	–
20	Geranyl acetate	$C_{12}H_{20}O$	1333	0.21	–	18.07	–
21	a-copaene	$C_{15}H_{24}$	1354	0.21	–	18.44	–
22	β-bourbonene	$C_{15}H_{24}$	1366	0.25	–	18.65	–
23	β-Elemene	$C_{15}H_{24}$	1378	0.17	–	18.87	–
24	α-Cedrene	$C_{15}H_{24}$	1399	0.68	–	19.23	–
25	β-caryophyllene	$C_{15}H_{24}$	1409	9.91	1.57	19.37	19.594
26	β-Cubebene	$C_{15}H_{24}$	1419	0.42	–	19.52	–
27	α-Bergamotene	$C_{15}H_{24}$	1425	8.33	0.94	19.61	19.81
28	Bicyclosesquiphellandrene	$C_{15}H_{24}$	1439	0.32	–	19.81	–
29	α-caryophyllene	$C_{15}H_{24}$	1447	1.38	0.12	19.94	20.129
30	epi-β-Santalene	$C_{15}H_{24}$	1452	0.60	0.33	20.01	22.489
31	Bicyclosesquiphellandrene	$C_{15}H_{24}$	1458	0.86	–	20.09	–
32	(1à,4aà,8aà)-1,2,3,4,4a,5,6,8a-Octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene	$C_{15}H_{24}$	1471	0.54	–	20.28	–
33	β-Cubebene	$C_{15}H_{24}$	1477	2.82	0.43	20.37	20.522
34	Elixene	$C_{15}H_{24}$	1492	2.27	–	20.59	–
35	a-Acoradiene	$C_{15}H_{24}$		–	0.24	–	20.738
36	l-b-Bisabolene	$C_{15}H_{24}$	1502	5.51	2.03	20.72	20.83
37	γ-Cadinene	$C_{15}H_{24}$	1511	0.63	–	20.83	–
38	δ-Cadinene	$C_{15}H_{24}$	1520	1.37	0.08	20.93	21.041
39	α-Muurolene	$C_{15}H_{24}$	1539	0.34	–	21.14	–
40	Espatulenol	$C_{15}H_{24}O$	1584	0.54	–	21.64	–
41	Caryophyllene oxide	$C_{15}H_{24}O$	1589	0.59	–	21.7	–
42	Spathulenol	$C_{15}H_{24}O$		–	0.13	–	21.711
43	Cedro	$C_{15}H_{26}O$	1612	0.23		21.94	
44	α-santalene	$C_{15}H_{24}$			0.13	–	22.056
45	5-hydroxy-2,3,3-trimethyl-2-(3-methylbuta-1,3-dienyl)cyclohexanone	$C_{14}H_{22}O_2$	1665	0.39	–	22.44	–
46	cis-α-Bisabolene	$C_{15}H_{24}$		–	0.41	–	22.728

–, not detected.
[a] HS-SPME headspace solid phase microextraction.
[b] UAHD ultrasound-assisted hydrodistillation.
[c] RT retention time.
[d] RI Retention indices, using paraffin (C7–C26) as references. RI reported only for the analytes present in Volatile compounds by HS-SPME.

Table 2
Antimicrobial activity (MIC and MBC/MFC values) of essential oil and its major components of bergamot.

Test microorganisms	Essential oil (μL/mL)		D-Limonene(μL/mL)		γ-Terpinene(μL/mL)		Antimicrobial agents(μg/mL)			
	MIC	MBC/MFC	MIC	MBC/MFC	MIC	MBC/MFC	MIC	MBC/MFC	MIC	MBC/MFC
Gram negative bacteria							Tetracycline Hydrochloride		Amphotericin B	
E. coli	0.78	1.56	50	> 50	50	> 50	7.5	30	NT[a]	NT
Gram positive bacteria										
B. subtilis	3.13	3.13	50	> 50	> 50	> 50	15	30	NT	NT
S. aureus	1.56	1.56	50	50	50	50	0.47	3.75	NT	NT
M. luteus	3.13	> 6.25	> 50	> 50	> 50	> 50	15	> 60	NT	NT
Yeast										
S. cerevisiae	1.56	1.56	6.25	6.25	50	50	NT	NT	3.75	> 15

[a] NT, not tested.

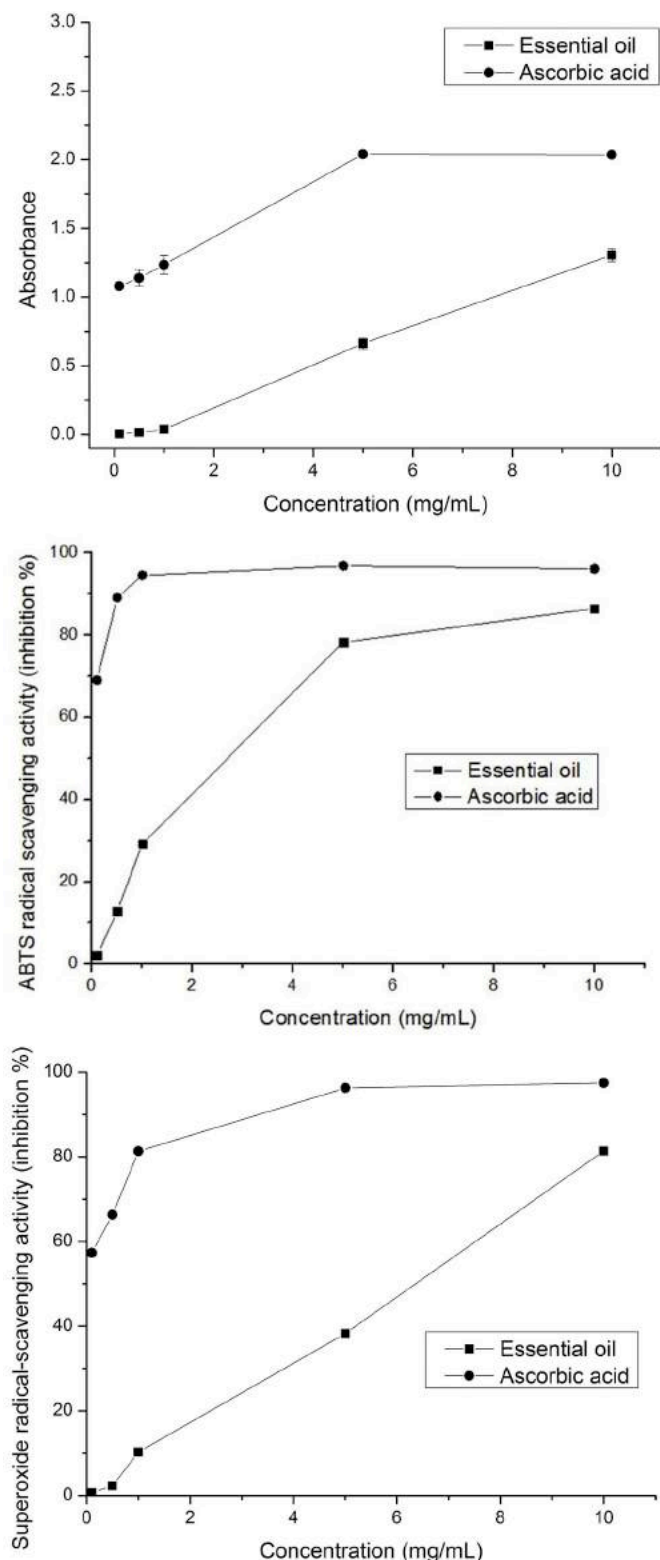

Fig. 3. Antioxidant potential of essential oil from the fruit of bergamot and ascorbic acid. A: Ferric reducing antioxidant power (FRAP) measurement; B: ABTS radical scavenging activity measurement; C: Superoxide radical-scavenging activity measurement.

concentration. Senthilkumar and Venkatesalu (2013) compared *F. limonia* fruit pulp essential oil with the same dose of BHT, the results showed that the IC50 (30.86 μg/mL) value of essential oil was lower than BHT (IC50 = 48.44 μg/mL) and had good antioxidant activity (Senthilkumar & Venkatesalu, 2013). The result of the present study is in agreement with the previous report that antioxidant properties of some essential oils are effective via scavenging of $O2^{-}$ radical.

4. Conclusions

A total of 46 compounds were identified from BEO, which was extracted by UAHD extraction process (118% higher than that of hydrodistillation). The main volatile components were found to be D-Limonene, γ-terpinene, β-caryophyllene, α-bergamotene and these compounds showed high antioxidant and antimicrobial activity. These results suggest that BEO might be used as a flavor product and also as a natural antioxidant and antimicrobial agent in the food, cosmetics and pharmaceuticals industries.

Acknowledgments

The study was supported by the National Natural Science Foundation of China (Grant No. 31671813) and Natural Science Foundation of Zhejiang Province, China (Grant No. LY17C200017).

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