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Chemical composition and antimicrobial activity of essential oils and extracts of two varieties of Turnip (Brassica rapa) root and leaves in Fars-Iran

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Abstract

The aim of this study was to evaluate the chemical composition of essential oil (EO) and antimicrobial activity of essential oils and extracts of two varieties of Turnip (*Brassica rapa*) root and leaves in Fars-Iran. The EO oil was obtained by hydro-distillation and analyzed by gas chromatography-mass spectrometry (GC-MS) which revealed 16 compounds in which methyl chavicol, transanethole, linalool and α-pinene were the main components. The antimicrobial activity was measured by disk-diffusion and micro-dilution method for determination of MIC and MBC. The highest inhibition zone was recorded against *Staphylococcus aureus* (33±2 mm) for EO of leaves from shirazi Turnip. The highest MIC (*S.aureus* and *Bacillus cereus* = 0.312 mg/ml) and MBC (*S. aureus*=0.625 mg/ml) values were detected for Shirazi Turnip leaves. Results presented suggest that the EOs and extract of turnip root and leaves possess antibacterial properties and potential application in food industry.

Key words : GC-MS, Turnip, Methyl chavicol, Anethole, Linalool, α-Pinene.

INTRODUCTION

Foodborne infections have been one of the major public health concerns worldwide and account for considerably high cases of illnesses [1]. Also the increasing antibiotic resistance of some pathogens associated with diseases has increased the interest in development of new types of effective and nontoxic antimicrobial compounds [2]. Addition of chemical preservatives has long been an effective method to control microbial contamination and development of oxidative reactions, although in recent years, popular demand has shown a negative vision to such synthetic chemical preservatives. This has resulted in a growing demand for natural products, principally, plant extracts, which are, in the consumers mind, safer, functional and provide nutritional and health benefits. This demand has increased the importance of searching for alternative sources of natural preservatives [3]. Use of natural antimicrobials such as organic acids, essential oils, plant extracts and bacteriocins could be a good alternative to ensure food safety [4].

Plant secondary metabolites such as essential oils and plant extracts, are studied for their antimicrobial activities and most essential oils derived from plants are known to possess antibacterial, insecticidal, antifungal, acaricidal and cytotoxic activities [5,6]. Essential oils (EOs) are complex mixtures of biologically active substances used for a long time as flavoring agents and constituents of a number of commercial products [7, 8]. The antimicrobial activity of EOs has created the opportunity to use them in pharmacological field and food preservation [9, 10].

B. rapa belongs to the Cruciferae (Brassicaceae) family, also known as the mustard family. The name Crucifer comes from the shape of flowers, with four diagonally opposite petals in the form of a cross [11]. This vegetable is usually grown in regions that experience temperate climates [12]. Turnip edible parts are commonly consumed as a boiled vegetable, being used in the preparation of soups and stews, too [13]. The antimicrobial activity of turnip was previously tested [14]. The highest antimicrobial activity was observed by methanolic extracts on Micrococcus species while mold was resistant to this extract. Other alcoholic extracts also showed such higher activity. Another research tried to examine the antibacterial effect of crude extracts of turnip species grown in northern Iran showed a significant antimicrobial activity of *B napus* L. against *pseudomonas aeruginosa* [15].Therefore, the aims of the present study were to examine the chemical composition of the EOs and to evaluate the antibacterial activities of EOs and extracts (aqueous and ethanolic) from leaves and roots of two varieties of *B. rapa* native to Fars province in Iran against food-borne pathogenic bacteria.

MATERIALS AND METHODS

Chemicals and plant materials

Ethanol and dimethyl sulfoxide (DMSO) (Merck, Germany) MHA (Muller Hinton Agar) and MHB (Muller Hinton Broth) (Merck, Germany) were purchased. Aerial parts (leaves) and roots of Turnip were collected in 2014 from Shiraz (GPS location: Latitude: 29° 36' 37.12" N, Longitude: 52° 31' 52.07" E) and Jahrom (GPS location: Latitude: 28° 56' 59.99" N, Longitude: 53° 56' 59.99" E) cities from Fars province in Iran. The plant was identified by voucher specimens (No.2383) in herbarium of department of biology, Shiraz university-Iran. The roots were cut into small pieces and dried in the shade also the leaves of plant were dried at shade [16].

EO preparation

The air-dried leaves and the roots of the plant (100 g) were powdered, and the EOs were isolated by hydro-distillation for 3 h using a Clevenger-type apparatus. The EOs were dried over anhydrous Na_2SO_4 and preserved in a sealed vial at 4ºC until further analysis [5, 17].

Preparation of extracts

Aqueous and ethanolic extracts of leaves and roots from Turnip were prepared by putting 50 g of each leave or root powder

in 250 ml of pure water [18] and ethanol. The mixture was agitated by orbital shaker for 24 h at 240 rpm and then was filtered over a Buchner funnel. The filtrates were put in oven at 40°Cafterward powder of extracts collected in sterile glass tubes [19, 20] and the yield of extraction was determined. All the dried extracts were preserved in refrigerator at 4ºC until further use. Plant extracts were dissolved in the solvent before use in the antimicrobial assay [21]. Dry matter contents of the extracts were in the range of 1.58-1.73% which varied between varieties.

Gas chromatography-mass spectrometry analysis

EOs were analyzed using an Agilent gas chromatograph Model 6890, coupled to an Agilent MS model 5973, equipped with a HP-5MS column (30 mm × 0.25 mm, coating thickness 0.25 µm) (Restek, Bellefonte, PA). Oven temperature program was from 50ºC (30 min) to 240ºC at 3/min. Helium (99.99 %) was the carrier gas at a flow rate of 1 ml/min. Diluted samples (1/100 in hexane, v/v) were injected manually [10]. Injector and detector temperatures were 220ºC. The electron impact mode, electron multiplier and ion source temperature of MS worked respectively at 70 eV, 1500 V and 240ºC [10,22].

The identification of the compounds was performed by matching their recorded mass spectra of the GC-MS data system. Quantitative data were obtained from electronic integration peak areas and comparing their retention time and mass spectra library with those found in the literature and supplemented by the Wiley (Wiley7th Mass Spectral Library) & NIST MS Search 2.0 (National Institute of Standards and Technology) GC-MS libraries [23].

Organisms and inoculation conditions

Four bacterial strains including *Staphylococcus aureus* (PTCC 1431), *Bacillus cereus* (PTCC 1212), *Salmonella typhimurium* (PTCC 1074) and *Escherichia coli* (PTCC 1330) were obtained from Persian Type Culture Collection, Iranian Research Organization for Science and Technology (PTCC, Iran). The bacterial strains were first grown on MH medium at 37°C for 24 h prior to seeding on to the nutrient agar [24]. Finally, suspensions were adjusted to 0.5 Mc-Farland standard turbidity. Bacterial suspensions were standardized to concentrations of 1.5×10^8 CFU/ml [25].

Antimicrobial assay

The antibacterial activity was studied using disk diffusion [26, 27] and micro-dilution methods [28]. All tests were performed in Triplicate.

Disk diffusion method

Sterile 6 mm filter paper disks [29] were placed on lawn culture prepared on Muller-Hinton (MH) agar (Merck) using sterile cotton swab impregnated with [30] 15 µl of [20] each bacterial suspension. The plates were put at room temperature for about 1 h to allow the extract and EO to diffuse from the discs into the medium, and then incubated at 37ºC for 24 h and after that the diameter of the zone from bacterial growth inhibition around each dick were measured and recorded in millimeter [30].

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Test

96-wellsmicro-plates were prepared by dispensing into each well with 100 µL of extracts and EOs. Then, 100 µL of MH broth [10] and 20µl of each bacterial suspension were added to the wells [31] (standardized at 1.5×10^6 CFU/ml by adjusting the optical density to 0.1 at 600 nm by Shimadzu UV-120-01 spectrophotometer). The final volume in each well was 220µl. The plates were covered with sterile plate sealer and followed by shaking for 30s and then incubated at 37°C for 24 h. After incubation, wells were examined for microbial growth. MIC was defined as the lowest concentration of the extract or essential oil in the medium in which there was no visible growth after incubation [32].Using the results of the MIC assay, the concentrations showing complete absence of visual growth of bacteria were identified and 50 µL of each culture broth was transferred on to the agar plates and incubated at 37ºC for 24 h. the complete absence of growth on the agar surface in the lowest concentration of sample was defined as MBC [33,34].

RESULTS

Chemical composition of Turnip EOs

The EOs were extracted by the hydro-distillation of the dried aerial parts (leaves) and roots of Shirazi and Jahromi *B. rapa* and the constituents were analyzed by GC-MS. The essential oil of *B. rapa* was analyzed to determine their constituents (Table 1).

GC/MS analyses showed 16 compounds in which the main components were Methyl chavicol (35.41%), trans-anethole (23.87%), linalool (18.32%), α-pinyin (9.28%) and β-pinyin and (5.11 %).

Disk diffusion results

Data are summarized in Table 2.All samples were virtually effective on pathogens especially essential oil of leaves which showed the largest inhibition zone on *S. aureus* (33±2 mm) and then on *B. cereus* (31±1 mm). The results indicated that essential oils at all tested concentrations significantly inhibited the growth of *S. aureus*, *B. cereus*, *S. typhimurium* and *E. coli*.

The Inhibition zone for ethanolic/aqueous extracts of Jahromi Turnip leaves and root are shown in table 2. All of the extracts had antimicrobial activity against tested bacteria. The inhibition zone of gram-positive bacteria were larger than gram-negative bacteria. These results coincided with the results obtained by Tenore et al. (2012) [35] and Beltagy et al. (2014) [15]. *B. cereus* was sensitive to ethanolic extract of leaves (16±0.6 mm) while aqueous extracts of leaves and root did not show strong antibacterial activity on *E. coli* (7±1 mm). Numerous surveys have highlighted the potential importance of extracts from *Brassica* species as sources of polyphenolics (flavonoids, phenolic acids and related analogues) able to exert antimicrobial effects [36, 37]. Actually, it is well known that phenolic acids are present in ionized form at the buffer H value (7.0) and are too polar to penetrate the semi permeable bacterial membrane and react with the cytoplasm or cellular proteins [38]. This is the same reason why the lipidic wall of gram-negative pathogens represents a great barrier for most polyphenols and hence only slight inhibition is achieved [15].

MIC and MBC data

Findings are presented in table3which shows the MIC values in the ranges of 3.125 (for *S. aureus* and *B. cereus*) and50 mg/ml (for *E. coli)*. Essential oils had higher inhibitory effects against all of the microorganisms. The MIC value was in range of 0.312-2.5 mg/ml and the essential oil of Shirazi Turnip leaves had the strongest bacteriostatic effect on the bacterial strains.

Table 4, reports the MBC values of the extracts and essential

Table 1: Chemical composition of turnip essential oils (%).

Component	EO from Shirazi Root	EO from Shirazi Leaves	EO from Jahromi Root	EO from Jahromi Leaves
α-Pinene	9.28	7.8	7.64	8.81
Linalool	15.46	18.32	13.96	17.65
β-Pinene	3.34	4.56	3.88	5.11
Cineol	1.39	0.68	1.24	0.57
Terpineol	0.8	0.6	1.05	0.82
Cis-Anethole	0.26	0.32	0.79	0.56
α-Thujone	6.59	3.41	4.46	3.54
Camphene	1.32	1.39	1.14	1.21
α-Terpinene	1.43	0.78	1.26	0.89
Limonene	2.01	2.51	1.83	2.12
Methyl chavicol	32.31	34.57	33.59	35.41
Trans anethole	19.58	21.19	23.87	20.52
Anisaldehyde	0.93	0.51	0.59	0.64
Anisic acid	1.56	0.49	0.83	0.37
Camazolen	0.46	0.18	0.39	0.28
Thymol	0.18	0.27	0.23	0.34
Total identified constituents	97.04	97.58	96.75	98.84

Table 2: Inhibition zone in diameter (mm) for ethanolic and aqueous extracts and EOs of Shirazi and Jahromi turnip leaves and root

Etracts/EO of leaves/root		*S. aureus*	*B. cereus*	*S. typhimurium*	*E. Coli*
Ethanolic extract of Shiraz	Leaves	18±1	16±1	14±1	13±1
	Root	14±1	15±0.57	11±1	10±0.57
Aqueous extract of Shiraz	Leaves	14±6	13±0.1	11±0.57	9±1
	Root	10±0.57	12±0.57	9±1	8±1
EOs of Shiraz	Leaves	33±2	31±1	26±1	22±1
	Root	26±1	26±2	23±1	17±1
Ethanolic extract of Jahrom	Leaves	16±0.57	16±0.60	12±1	12±1
	Root	12±1	13±1	10±0.57	8±1
Aqueous extract of Jahrom	Leaves	11±0.57	10±0.57	9±0.57	7±1
	Root	9±1	9±1	7±1	7±1
EOs of Jahromi	Leaves	25±1	23±2	21±1	19±1
	Root	21±2	19±1	18±2	15±1

Values are the mean of triplicate.

oils against bacterial strains which were prevented at different rates. The most sensitivity was observed for *Bacillus cereus* and *S. aureus* and the least for *S. typhimurium* and *E. coli*. The MBC value was between 0.625-50 mg/ml.

DISCUSSION

The antimicrobial activity of extracts obtained from spices, herbs and other aromatic plants have been recognized for many years [39]. Various publications have documented the antibacterial activity of essential oil constituents and plant extracts [31, 40, 41]. In this study, the essential oil and extracts exhibited remarkable activity against some of the representative food borne and spoilage pathogenic bacteria such as *S. aureus, B. cereus*, *S. typhimurium, E. coli.* The Gram-positive bacteria were found to

Table 3: MIC for ethanolic and aqueous extracts and EOs of Shirazi and Jahromi turnip leaves and root (mg/ml).

Etracts/EO of leaves/root		*S. aureus*	*B. cereus*	*S. typhimurium*	*E. Coli*
Ethanolic extract of Shiraz	Leaves	3.125	3.125	6.25	12.5
	Root	6.25	6.25	12.5	12.5
Aqueous extract of Shiraz	Leaves	6.25	6.25	12.5	12.5
	Root	6.25	6.25	25	25
EOs of Shiraz	Leaves	0.312	0.625	1.25	1.25
	Root	0.625	1.25	2.5	2.5
Ethanolic extract of Jahrom	Leaves	6.25	6.25	25	25
	Root	12.5	12.5	25	25
Aqueous extract of Jahrom	Leaves	12.5	12.5	25	25
	Root	12.5	12.5	25	50
EOs of Jahromi	Leaves	0.625	1.25	1.25	5
	Root	1.25	1.25	2.5	2.5

Values are the mean of triplicate.

Table 4: MBC for ethanolic and aqueous extracts and EOs of Shirazi and Jahromi turnip leaves and root (mg/ml)

Etracts/EO of leaves/root		*S. aureus*	*B. cereus*	*S. typhimurium*	*E. Coli*
Ethanolic extract of Shiraz	Leave	6.25	6.25	6.25	12.5
	Root	12.5	12.5	25	25
Aqueous extract of Shiraz	Leave	6.25	6.25	12.5	25
	Root	12.5	12.5	50	50
EOs of Shiraz	Leave	0.625	0.625	2.5	2.5
	Root	1.25	2.25	5	5
Ethanolic extract of Jahrom	Leave	6.25	25	25	50
	Root	25	25	50	50
Aqueous extract of Jahrom	Leave	25	12.5	25	50
	Root	25	25	50	50
EOs of Jahromi	Leave	1.25	1.25	2.5	5
	Root	2.5	2.5	5	5

Values are the mean of two replicate.

be more susceptible to the essential oil and various solvent extracts than Gram-negative bacteria. This is probably due to the cell membrane of Gram-positive bacteria, which can interact directly with hydrophobic compounds of essential oils, whereas the external cell wall around the cell membrane of Gram-negative bacteria is hydrophilic and blocks the penetration of hydrophobic oil and avoids the accumulation of essential oils in target cell membrane [31,42].

Regarding the antibacterial activity of plant extracts/essential oils, the researchers have described several mechanisms of action, including cell membrane damage resulting in increased permeability, changes in intracellular pH and membrane potential, dissipation of cellular components, decrease in the cytoplasmic ATP concentration, which together induce bacterial death. Secondary effects that may be involved seem to be the inhibition of enzymes, loss of turgor pressure, alterations in macromolecules synthesis, and other cellular processes [43].

CONCLUSION

The results presented in this study indicated that essential oils and extracts obtained from leaves and roots of turnip (*B. rapa)* possess antibacterial properties. On the basis of the experimental results, it can be postulated that the extracts of turnip (*B. rapa)*

have the potent antibacterial properties against some representative food-borne pathogens. Specifically, turnip leaves essential oils and extracts obtained by alcoholic solvent which presence of more phenolic compounds than others is reason. Therefore, they could be used as possible food antimicrobial preservative in food industry, but the in vivo studies should be done to evaluate the probable adverse effect on food sensory properties.

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