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Research Article

Antibacterial and Antioxidant Activities of the Volatile Composition of the Flower and Fruit of *Solanum sisymbriifolium* (Litchi Tomato)

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ABSTRACT

Background: *Solanum sisymbriifolium* Lam. is used as traditional remedy in South America, recently this plant considered as new edible source. Berries and flower of *S. sisymbriifolium* have a characteristic fragrance. The pleasant fragrance of the *S. sisymbriifolium* could be considered as a source of food additive or preservative.

Methods: The essential oils of the flower and fruit of *S. sisymbriifolium* Lam. (litchi tomato) were isolated by hydrodistillation method and tested for antibacterial and antioxidant potentials also these volatile oils analyzed by the gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detection (GC-FID).The antimicrobial activity of the essential oils of fruits and flowers were tested against *Staphylococcus aureus* using the well diffusion method and their free-radical-scavenging activity were assessed by the 2, 2-diphenyl-picryl-hydrazyl (DPPH) assay.

Results: The essential oil of flower was characterized by a high content of aldehydes and aliphatic hydrocarbons (66.8%) and the essential oil of the fruit has high amount of fatty acids and their derivatives (80.1%). Heptadecane (37.9 %) and 9,12,15-octadecatrienal (22.7%) were the main compounds in flower whereas the fruits essential oil contained hexadecanoic acid (77.4%) and ambrettolide (7.4%). The essential oils showed antibacterial activity against *S. aureus* in 60 and 80 µg/mL for fruit and flower, respectively. In antioxidant activity assay fruit essential oil (with100 µg/mL) showed better activity in compression to flower essential oil with 83.33% activity.

Conclusion: This study showed that litchi tomato can be considered as a new source of edible compounds. Flower showed suitable antioxidant and antibacterial activity. This study also can be present an overview about chemical marker compounds of *Solanum* genus.

Introduction

Essential oils have been shown antibacterial, antifungal, antiviral, insecticidal and antioxidant properties. Among of the biological activities, antibacterial and antioxidant activities of the essential oils have been known for a long time, many of plants and their volatile oils used traditionally as a disinfectant in many countries also some essential oils have been used in food preservation.[1-5] Some essential oils such as cinnamon, clove, and rosemary oils had antibacterial and antioxidant activities. Therefore essential oils are a potential source of biologically active compounds. The essential oils (or volatile oils) obtained from plant materials such as flowers, seeds, leaves, bark, wood, fruits and roots by expression, distillation, and solvent extraction methods.[6-9] Essential oils composition of every plant is a variable ratio of many single compounds that chemically derived from terpenes, fatty acids, aliphatic hydrocarbons and oxygenated compounds. This compound is one of the natural antioxidants constitute that including abroad range of phytochemical. Among the *Solanaceae* family,

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common secondary metabolites are terpenes, fatty acids, and alkaloids. Many biological activities especially antibacterial and antioxidant have been reported until now but negligible researches have been conducted on essential oils, biological activities and nutraceutical potencies this genus.[10-12] Fragrant fruits of *S. sisymbriifolium* (litchi tomato or sticky nightshade) used as a traditional remedy in South America countries. However, no information existed about the essential oil profile, antioxidant and antibacterial activities of *S. sisymbriifolium* but most experimental studies showed that this genus has proper chemical constitutions which act as an antibacterial and antioxidant agents. Therefore, the aim of the present study was to evaluate antibacterial and antioxidant activities of the flower and fruit of *S. sisymbriifolium* essential oils as edible vegetable and folk remedy.

Material and Methods

Plant Material

Flower and fruit of *S. sisymbriifolium* were collected from awild population growing in Guillan province, Iran. Voucher specimens (Nos 2560) were authenticated by the pharmacognosy department in the herbarium of pharmacognosy department of pharmacy faculty of Guilan University of Medical Sciences,Rasht, Iran.

Extraction of the Essential oil

The air dried flower and fruit of *S. sisymbriifolium* (500 gr) were subjected to hydrodistillation for 3 h in a Clevenger-type apparatus. The essential oils extraction out put was 0.8% and 1.2% v/w for fruit and flower respectively. Yellowish oils (by dry mass) with a fragrant smell. The essential oils were dried over anhydrous sodium sulfate (Na_2SO_4) and stored at 4 ˚C in the dark until tested and analysis by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS).

Analysis of the Essential oil

The essential oils were analyzed by Shimadzu GC-MS-QP5050A fitted with a fused methyl silicon DB-5 column (60 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was used as carrier gas at a flow rate of 1.3 mL/min. The column temperature was kept at 50 °C for 3 min, increased to 300°C at a rate of 5°C/min, and finally kept at 300 °C for 5 miniutes. The injector temperature was 270ºC and split ratio was adjusted at 1:33. The injection volume was 1 µL. The mass spectral (MS) data were obtained at the following conditions: ionization potential 70 eV; ion source temperature 200 °C; quadrupole temperature 100 °C; solvent delay 2 min; resolution 2000 amu/s and scan range 30-600 amu; EM voltage 3000 volts. Identification of compounds was based on direct comparison of the Kovats indices and MS data with those for standard compounds, and computer matching with the National Institute of Standards and Technology (NIST) NBS54K Library, by comparison with references. For quantization (area %), the GC analyses were also performed on an Agilent 6890 series apparatus fitted with a FID detector. The FID detector temperature was 300 ºC. To obtain the same elution order as with GC-MS, the simultaneous auto-injection was performed on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

The DPPH assay

The free-radical-scavenging property of the essential oil was assessed using the DPPH (2, 2diphenyl-1-picrylhydrazyl) assay as described in the literature.[13-15] DPPH was purchased from Fluka Chemie AG, Bucks. 4 mg DPPH was dissolved in 50 ml methanol to prepare DPPH solution (80 µg/mL).Various concentrations of each essential oils (10, 40, 60, 80,100 µg/ml) were add to DPPH solution. These mixtures were shaken and allow standing for 30 min at room temperature. The absorbance of these samples measured at 517 nm for flower and root essential oil samples. Trolox used as a standard antioxidant and all experiments were repeated three times.

Antibacterial assay

The bacterial culture of *Staphylococcus aureus* (ATCC 6538) was used to evaluate the antibacterial properties of the essential oils using the well-diffusion method.[16] Bacterial cells were purchased in lyophilized form from the Institute of Pasture, Iran. These were cultured in LB agar medium after dissolving in sterile distilled water. The plates were incubated at 37°C for 24h. A single colony from the plate was transferred into 4 mL fluid LB medium and incubated overnight at 37°C and 200 rpm in shaking incubator. The cells were harvested by centrifugation at 3000 rpm for 15 min and 4˚C. Subsequently, they were washed twice and re-suspended in Ringer solution to provide bacterial concentrations between 10^6-10^7cfu/mL.[17] The medium was inoculated with the microorganism. Once the agar was solidified, it was punched with a six millimeters diameter wells and filled with 25 µL of the essential oil of various concentrations (10-100 µg/mL) and blank. Simultaneously ampicillin was used as a positive control at a concentration of 10 µg/mL. The dilution medium for the positive controls was sterile distilled water. The test was carried out in triplicate. The plates were incubated at 35 ± 2°C for 24 h. The antimicrobial activity was calculated as the zones of inhibition in mm.

Table1. Chemical compositions of the essential oil of flower and fruit of *S. sisymbriifolium.*

	Flower		Fruit	
Compounds [a]	**RI[b]**	**Percentage in sample (%)**	**RI[b]**	**Percentage in sample (%)**
3-Hexen-2-one	956	0.5	-	-
Hemimellitene	-	-	995	0.2
Alpha-terpinolene	1084	0.2	-	-
2-Hexanoylfuran	1091	0.1	-	-
Dodecane	-	-	1118	1.3
10-Undecyn-1-ol	1175	0.4	-	-
Tridecane	1296	0.1	-	-
Delta-cadinene	1336	0.7	-	-
Alpha-bourbonene	1351	0.2	-	-
Isoledene	1362	0.2	-	-
2-Carene, 4-.alpha.-isopropenyl	-	-	1378	0.7
Beta-elemene	1384	0.2	-	-
Beta-caryophyllene	1403	0.1	-	-
Neryl acetone	1428	1.3	-	-
1-Tetradecen-3-yne	1436	0.2	-	-
Germacrene D	1476	3.3	-	-
Alpha-farnesene	1506	0.1	-	-
Alpha-Copaene	1509	0.1	-	-
Hexadecane	1589	0.4	-	-
9-Tetradecenal	1601	0.7	-	-
tricyclo[5.2.2.0(1,6)]undecan-3-ol,2-methylene-6,8,8-trimethyl	1609	0.1	-	-
Tetradecanal	1640	0.7	-	-
1-Hydroxy-6-(3-isopropenyl-cycloprop-1-enyl)-6-methyl-heptan-2-one	-	-	1654	5.1
Tetradecanoic acid	-	-	1689	0.6
Tetradecanoic acid	1706	0.2	-	-
Hexadecanal	1814	0.3	-	-
Pentadecanoic acid	-	-	1820	1.1
Farnesyl methyl ester	1853	6.22	-	-
2-methyloctadecane	1864	0.1	-	-
9-Nonadecene	1896	0.1	-	-
Ambrettolide	1952	0.3	1952	7.4
Hexadecanoic acid	1965	10.6	1965	77.4
Heptadecane	1989	37.9	-	-
9,17-Octadecadienal	1993	0.2		-
Eicosane	2000	1.4	-	-
Octadecanal	2006	0.4	-	-
9,12,15-Octadecatrien-1-ol	2082	5.3	2096	1.7
Methyl linolenate	2090	0.3	-	-
9,12,15-Octadecatrienal	2096	22.7	-	-
Nonadecanone	2097	0.8	-	-
Phytol	2115	0.1	-	-
Octadecanoic acid	2126	0.1	-	-
9-octadecenoic acid	-	-	2141	1.0
1-Eicosanol	2273	0.1	-	-
11,14-Eicosadienoic acid, methyl ester	2289	0.5	-	-
1-Docosene	2200	0.3	-	-
Total		97.4		96.5
Aldehydes hydrocarbons (%)		26.2		-
Fatty acid and derivatives (%)		18.4		80.1
Sesquiterpene hydrocarbons (%)		5.5		-
Aliphatic hydrocarbons (%)		40.6		-
Isopropenyl derivative (%)		-		5.8

Table 1 Continued.

Others (%)	6.7	10.6

[a]Compounds are listed in order of their elution from a DB-5 column.
[b]RI= retention indices as determined on DB-5 column using homologous series of *n-alkanes.*
[c]Percentage obtained by FID peak normalization; values represent an average of three determinations.

Results and Discussion

A total of thirty-nine compounds, representing about 97.4% of the total oil, were identified in the essential oil of *S. sisymbriifolium* flower. Heptadecane (37.9%) and 9, 12, 15-octadecatrienal (22.7%) constituted almost two third of the fruit essential oil. Among the others constituents hexadecanoic acid (10.6%), methyl farnesoate (6.2%) and 9, 12, 15-octadecatrien-1-ol (5.3%) were notable. The essential oil of the fruit characterized by high content of hexadecanoic acid (77.4%) followed by ambrettolide (7.4%) and 1-hydroxy-6-(3-isopropenyl-cycloprop-1-enyl)-6-methyl-heptan-2-one (5.1%) (Table 1).It appears that the compositions of flower essential oil are different from the fruit oil compositions that mainly represented with sesquiterpene and aldehydes hydrocarbons. However, the same profile of some chemical compounds such as ambrettolide, hexadecanoic acid and 9, 12, 15-octadecatrien-1-ol is evident in flower and fruit of *S. sisymbriifolium*.

very limited studies conducted on the essential oils of *Solanaceae* family, therefore negligible knowledge exist about their constituents and bioactivity of them. Investigation on *Lycium barbarum* and *L. ruthenicum* (*Solanaceae*) fruits identified that fatty acids such as hexadecanoic acid, linoleic acid as well as unbranched alkanes constitute the major compounents of the essential oil.[18] In similar work on compositions of *L. chinense* Mill. essential oil indicated that fatty acids and fatty acid esters such as hexadecanoic acid methyl ester , hexadecanoic acid ethyl ester, tetradecanoic acid and hexadecanoic acid formed the major components of the fruit essential oil.[19] In previous research on this plant determined that *β*-ionone derivatives such as 3-hydroxy-7, 8-dihydro-*β*-ionone, 3-hydroxy7, 8-dihydro-*β*-ionol, 3-hydroxy-*β*-ionone and 3-hydroxy*β*-ionol consist the major portion of the leaves essential oil that this finding clearly showed difference between fruit and leaves essential oils compositions.[20] Another investigation on difference of berries and leaves essential oils content of *Solanum sarrachoides* showed that monoterpenes and fatty acids are the main compounds, however, the relative abundance of them different between berries and leaves essential oil. camphor, limonene, decanoic acid, and hexadecanoic acid making up the main constituents of the leaf, In contrast, the berry essential oil mainly include hexadecanoic acid, dodecanoic acid, and unbranched hydrocarbons.[21] Other research on *Solanum spirale* Roxb. Leaves essential oil showed that (*E*)-Phytol, *n*-hexadecanoic acid, β-selinene, and α-selinene were the major components of essential oil. The essential oil of this spices showed significant antibacterial activity against *E. coli* and *S. aureus* (MIC 43.0 and 21.5 µg/mL) also this essential oil exhibited antioxidant activity in DPPH assay with IC_{50} of 41.89 mg/mL.[22] Future works on other plants, *Cestrum diurnum* (Solanaceae) showed that potential of the essential oil of this plant especially against *P. aeruginosa* and *S. aureus*. Palmitic acid, stearic acid, and oleic acid were constituted the main components in the essential oil of the leaves of this plant.[23]

The antioxidant activities of essential oils in different concentration have been evaluated by the DPPH scavenging activity assay (Table 2). It showed that flower essential oils displayed considerable free radical scavenging activity comparable to the fruit and positive control Trolox (Table 2). The potential of free-radical scavenging activity of the flower essential oil was mainly owing to the presence of high amount of unsaturated compounds such as 9,12,15-octadecatrienal, methyl farnesoate and 9,12,15-octadecatrien-1-ol.[24,25]

Table 2. Antimicrobial and DPPH scavenging activities of the flower and fruit essential oil of *S. sisymbriifolium.*

Antimicrobial activity	Antimicrobial assay (zone of inhibition in mm)		DPPH scavenging activities	Antioxidant activity (inhibition %)	
Conc.(µg/mL)	**Flower**	**Fruit**	**Conc.(µg/mL)**	**Flower**	**Fruit**
10	-	-	10	10.25 ± 2.35	5.68±1.15
40	-	-	40	25.31 ± 1.12	13.58±2.60
60	8	n/a	60	49.46 ± 4.65	53.74±1.82
80	10	6	80	74.22 ± 7.64	69.42±3.70
100	15	10	100	83.33 ± 5.35	54.01±1.23
*Ampicillin	21	21	*Trolox	97.62 ± 3.14	97.62±3.14

*Positive controls; N/A = Not applicable

This is the first report on the antibacterial activity of the essential oils of this species. In antibacterial assay result, flower essential oil showed comparable antibacterial activities to the ampicillin (10 µg/mL) against *Staphylococcus aureus* in 100 µg/mL (Table 2). According to the previous finding, the essential oils with high content of aldehydes and sesquiterpenes hydrocarbons showed suitable antibacterial effects[26-28] that the current finding is in line with the previous results.

Conclusion

The present study showed that differences between the flower and fruit essential oil of *S. sisymbriifolium* composition, antibacterial, and antioxidant activities of them. Better antibacterial and DPPH scavenging activities of the flower essential oil may be as a result of the major compounds such as unsaturated compounds, sesquiterpene, and aldehydes. This is the first report on essential oil compositions, antioxidant and antibacterial activities of *S. sisymbriifolium.*

Conflict of interests

The authors claim that there is no conflict of interest.

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