SAMBUCUS EBULUS, INTRODUCTION TO MECHANISM OF ACTION; A CHEMICAL VIEWPOINT

Feizbakhsh A.[1], Pazoki H.[1], Ebrahimzadeh M.A.[2*]

[1]Department of Chemistry, Islamic Azad University, Central Tehran Branch, Tehran, Iran.
[2]Pharmaceutical Sciences Research Center, School of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran. Tel.: +98 151 3543081-3; Fax: +98 151 3543084. E-mail: zadeh20@yahoo.com

Summary

Essential oil composition of *Sambucus ebulus* leaf of *(Caprifoliaceous)* was analysed by GC and GC–MS. Sixty compounds were identified. The major components were β-bisabolene (11.4%), germacrene D (6.9%), geranyl acetate (5.6%) and α-cubebene (5.2%). The content of total phenolic compounds and flavonoids were also measured. Some detected compounds can be responsible for plant biological and/ or toxic activities.

Key Words: *Sambucus Ebulus*; essential oil; β-Caryophyllene; α-Thujone.

Introduction

Four species of the genus *Sambucus* are growing in Iran. Of these species, *S. ebulus* (*Caprifoliaceous*) extensively grows in the northern regions of Iran (1,2). Iranian traditional medicine uses, in variousoccasions, the leaves and rhizomes of *S. ebulus* in treating some inflammatory cases such as, bee and nettle bites, arthritis, and sore-throat (3). In addition, it has been reported to be an insect repellent, *anti*-hemorrhoid, anti bacterial toward *Helicobacter pylori*, useful in the treatment of burns and infectious wounds, edema, eczema, urticaria, the cold, inflammation and rheumatism (2,4,5). Despite sporadic references on the activity of SE, there exist little or no systematic records on the use of SE as a widely accepted medicinal plant in Iran. Recently a significant anti-inflammatory activity was reported (1,2). Flavonoids, steroids, tannins, glycosides, cardiac glycosides, caffeic acid derivatives, ebulitins, volatile substances, phenol and flavenoid content of this species was previously reported (1,6). Recently we have reported good antioxidant activity of SE fruit and flowers (7,8). Studies have established extracts of some plants which are rich in flavonoids (9) and many of these phenolic compounds have shown to be cytoprotective by reducing oxidative stress (10,11), thereby giving a solid basis to the proposal that the antioxidant content of plants could account for its cardio-protective properties (12). Assuming its therapeutic benefit, it is attributed to antioxidant activity, we have reported antioxidant potential of fruit and flowers extracts of *S. ebulus* (7,8). For this purpose, and in continuation of our research, total phenol and flavonoids contents of *S. ebulus* leaf extract were measured. To the best of our knowledge, no information is available about the composition of the *S. ebulus* leaf essential oil from Iran. In this study, chemical composition of volatile oil also was evaluated. It seems some compounds in leaf composition can play a role in interpretation of mode of action in some biological and/or toxic activities that have been reported previously from *S. ebulus*.

Materials and methods

Chemicals: Solvents and fine chemicals were purchased from Merck or Fluka Companies and were of highest purity and analytical grade. Leaves were collected and dried as stated below.

Plant material, preparation of extract and essential oil composition: Fresh leaves of *S. ebulus* were collected from Mazandaran forest in August 2009. After identification of the plant by Dr B. Eslami a voucher (No. 211) has been deposited in the Faculty of Pharmacy herbarium. Leaves were dried at room temperature and coarsely ground before extraction. Air dried leaves (100g) were extracted by percolation method using methanol (300 ml) for 24 h at room temperature. The extract was then separated from the sample residue by filtration through Whatman No.1 filter paper. This procedure repeated three times. The resultant extracts were concentrated in a rotary evaporator until a crude solid extract was obtained. Yield was 31%. In addition, the air-dried leaves were subjected to hydrodistillation, using a Clevenger-type apparatus for 4 h. The oil was dried over anhydrous sodium sulphate and stored in a sealed vial at low temperature before analysis (0.1%). The oil was analyzed by GC/MS analysis.

GC/MS: The Oil was analyzed by GC/MS using a Hewlett Packard 5973N mass selective detector connected with an HP 6890N gas chromatograph. The separation was achieved by capillary column, HP-5 MS (30 m×320 μm). The column temperature was kept at 60 °C for 20 min and programmed to 220 ^{o}C at a rate of 5 ^{o}C/min, and kept constant at 220 ^{o}C for 20 min. Injector temperature was 270 ^{o}C. Helium was used as carrier gas at a flow rate of 1 ml/min. MS was taken at 70 eV. The components of the oil were identified by their retention times, retention indices relative to C9–C28 *n*-alkanes, computer matching with the AUTOINT 1. E library and comparison of their mass spectra with those of authentic samples or with data already available in the literature (13-16). The percentage of composition of the identified compounds was computed from the GC peak area without any correction factor and was calculated relatively.

Total flavonoids determination: Flavonoid content of extract was determined by following colorimetric method (17,18). Briefly, 0.5 ml solution of extract (at 10% w/v) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/ Visible spectrophotometer (UV- Visible EZ201, Perkin Elmer: USA). The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 μg ml^{-1} in methanol.

Total phenols determination: Total phenols were determined by Folin Ciocalteu reagent (19,20). Briefly, 0.5 ml solution of extract (at 10% w/v) or gallic acid (standard phenol compound) in methanol were mixed with 5 ml of Folin Ciocalteu reagent (a 10% v/ v in distilled water) and 4 ml of 1 M aqueous Na_2CO_3. The mixtures were kept for 15 minutes and the total phenol content were determined by colorimeter at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mgL^{-1} solutions of gallic acid in methanol: water (50:50). Total phenol values are expressed in terms of gallic acid equivalent (mg/g of dry mass) which is a common reference compound.

Results and discussion

Total Phenol and Flavonoid Contents: Total phenolic compounds, measured by Folin Ciocalteu reagent, are reported as gallic acid equivalents by reference to standard curve (y= 0.0063x, r^2= 0.987). The total phenolic contents of *S. ebulus* leaf were 27.40± 0.8 mg gallic acid equivalent/g of extract. The total flavonoid contents of *S. ebulus* was 24.65± 0.5 mg quercetin equivalent/g of extract, by reference to standard curve (y= 0.0067x+ 0.0132, r^2= 0.999). Extract contained high level of total phenol and flavonoid contents. Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (21). The antioxidant activity of quercetin and other flavonoid previously reported (22-24). Some part of activity of *S. ebulus* maybe result of its high flavonoid content. In addition, anti-inflammatory activity of gallic acid (25) and quercetin (26) reported previously and good anti-inflammatory activity of *S. ebulus* maybe results of presenting of these compounds.

Chemical composition of the essential oil: The results obtained by the GC-MS analysis of the essential oil of the *S. ebulus* leaf are presented in Table 1. Sixty compounds were identified, representing 97.33% of the total oil. The oil yield of the plant was determined as 0.1% v/w. As determined from the GC-MS analysis, the major compounds were β-bisabolene (11.4%), germacrene D (6.9%), geranyl acetate (5.6%) and α-cubebene (5.2%). α-Thujone is the toxic monoterpenoid in some herbal medicines and is reported to have antinociceptive, insecticidal and anthelmintic activity (27). The acute toxic effect of α-thujone consists of convulsions (28). Long-term ingestion of plants containing this compound can cause hallucinations, sleeplessness, tremor, convulsions, and paralysis, a syndrome called absinthism (28). Animal experiments have shown that α-thujone is neurotoxic (28). The presence of α-Thujone in leaf composition can improve some plant′s toxicity and biological activities we have reported recently (1,2). Eugenol can inhibit prostaglandins synthesis (29). Eugenol and isoeugenol are well known to possess antioxidant activity (30). There have been many studies on the cytotoxicity of o-methoxyphenols such as eugenol and isoeugenol (31). These molecules possess prooxidant as well as antioxidant activities (31) under certain circumstances. In general, low concentrations of eugenol are thought to as antioxidants, with beneficial anti-inflammatory effects; whereas high concentrations act as prooxidants, leading to tissue damage as a result of the formation of harmful phenoxyl radicals (31). Eugenol has been long known for its analgesic, local anesthetic, anti-inflammatory, and anti- bacterial effects (32, 33). In addition, eugenol is thought to be antipyretic (34). The presence of eugenol and isoeugenol in leaf composition can improve plant′s antioxidant, anti-inflammatory and anti-noceceptive properties we have reported recently (1,2,7,8). Germacrene D is a sesquiterpene hydrocarbon (35). In plants, the volatile sesquiterpene hydrocarbons themselves are well known as constituents of essential oils and have evolved ecological roles in the interaction of the plant with insects and other predators and pollinators. The effects of germacrene D on insect behavior have been documented in the literature (36). Bisabolene, geranial and borneol have recently been studied and found to possess beneficial properties for the treatment of poor digestion, heartburn, vomiting and preventing motion sickness (35). β-Caryophyllene is a bicyclic sesquiterpene that is quite widely distributed in plants (37). It possesses anticarcinogenic activity (38) also could play a role in plant defense (37). It has been used as natural remedies and as fragrances (34). The odor of β-caryophyllene has been commonly used as a fragrance chemical since the 1930s (40). Caryophyllene was detected in 33% of 300 analyzed cosmetic products on the Dutch market in the beginning of the 1990s (34). It is used in spice blends, citrus flavors, soaps, detergents, creams and lotions, and also in a variety of food products and beverages (41). β-Caryophyllene is known for its anti-inflammatory

and local anaesthetic activities (42-44). The presence of β-Caryophyllene in leaf composition can improves plant′s anti-inflammatory activity we have reported recently (1,2).

Table1. Chemical composition of the essential oil of *S. ebulus* leaf.

No	Compound	KI	%	No	Compound	KI	%
1	β-Pinene	976	0.57	31	Piperitone	1286	1.24
2	α-Terpinene	1012	0.28	32	cis-Pinocarvyl acetate	1293	1.24
3	Linalool oxide	1062	0.74	33	Myrtenyl acetate	1300	1.05
4	α-Thujone	1094	1.19	34	trans-Verbenol	1306	3.74
5	β-Thujone	1101	0.81	35	Trans Carvyl acetate	1318	2.32
6	Ocimene oxide	1110	1.16	36	Eugenol	1332	2.05
7	Ocimenone	1124	0.38	37	δ-Elemene	1339	2.32
8	Camphor	1129	0.76	38	α-Cubebene	1358	5.22
9	iso Pulegol	1135	0.09	39	Geranyl acetate	1363	5.65
10	Pino carvone	1140	0.23	40	α-Bourbonene	1376	3.85
11	iso Borneol	1147	0.92	41	α-Copaene	1379	1.88
12	Borneol	1156	0.58	42	β-Cubebene	1388	0.29
13	Terpinen-4-ol	1167	2.39	43	iso-Longifolene	1392	1.93
14	Myrtenal	1174	0.33	44	Cyperene	1404	3.13
15	α-Terpineol	1178	1.45	45	Longifolene	1408	0.28
16	Myrtenol	1184	0.64	46	β-Gurjunene	1412	0.84
17	Verbenone	1191	1.19	47	β-Caryophyllen	1418	2.14
18	Fragranol	1196	0.44	48	β-Caryophyllen oxide	1425	3.28
19	trans Carveol	1200	0.27	49	γ-Elemene	1434	0.99
20	cis Carveol	1211	3.86	50	Aromadendrene	1440	1.77
21	Pulegol	1215	0.11	51	Dehydro aromadendrane	1458	0.72
22	Carvone	1218	0.5	52	Germacrene D	1480	6.89
23	Pulegone	1220	0.98	53	β-Selinene	1483	0.66
24	Chavicol	1229	2.01	54	epi-Cubebol	1491	0.25
25	Geraniol	1236	1.52	55	β-Bisabolene	1507	11.41
26	Geranial	1245	0.95	56	Cubebol	1513	0.81
27	iso Estragol	1260	1.39	57	δ-Cadinene	1521	1.66
28	Safrol	1269	0.19	58	Germacrene B	1557	0.18
29	Bornyl ac	1272	0.92	59	Caryophyllen epoxide	1565	0.51
30	Carvacrol	1281	0.72	60	Caryophyllenol I	1650	1.45

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