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EFFECT OF GIBBERELLIC ACID ON COMPOSITION OF *S. EBULUS* LEAF ESSENTIAL OIL (CAPRIFOLIACEOUS)

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.

[*]zadeh20@gmail.com

Abstract

Plant hormones control virtually all aspects of plant development. In this study the effects of growth hormone, Gibberellic acid (GA3), on essential oil of *Sambucus ebulus* (Caprifoliaceous) leaf were evaluated. Water distillation method was used for extracting the essential oil by Clevenger apparatus and then was analyzed by capillary GC and GC/MS. Sixty constituents were identified in the plant oil. Some detected compounds can be responsible for the plants biological and/ or toxic activities. Results indicate that GA3 has significant effect on the concentration of essential oil. Many components were increased and some of them decrease significantly. In some cases, the compounds were eradicated of induced completely. It seems a useful method for changing the concentration of the essential oil compounds.

Key words: Caprifoliaceous, Essential oil, Gibberellic acid, Phytohormones, *Sambucus Ebulus*.

Introduction

Phytohormones are small organic molecules, commonly used to increase grain production [1]. Phytohormones are regulators produced by plants themselves, which control the physiological processes. As a minor component of the metabolome, phytohormones are of particular significance given their role in the regulation of germination, growth, reproduction, and the protective responses of plants against stress [2]. Gibberellic acid (GA3) is the chief representatives of gibberellins [3]. The quantitative analysis of phytohormones is often required in agriculture and plant physiology. Several methods have been used for the analysis of phytohormones. Mass spectrometry (MS) is the most powerful detector for the determination of phytohormones due to its high sensitivity and selectivity. Gas chromatography coupled with mass spectrometry (GC-MS) [4] was also reported. Gibberellic acid (GA3), accelerates and improves the yield of a wide variety of plants by increasing cell division [5,6]. Genus of Sambucus belongs to Caprifoliaceae and included eighteen species in all over the world, among them six species distributed in subtropical areas of America, Eurasia and Africa. Four species of the genus Sambucus are growing in Iran. Of these species, S. ebulus extensively growth in moist grasslands or forest margins on Northern coast of Caspian Sea. Iranian traditional medicine uses the leaves, fruits and rhizomes of S. ebulus in treating some inflammatory cases such as, bee and nettle bites, arthritis, and sore-throat [7,8]. In addition, it has been reported to be an insect repellent, antihemorrhoid, antiprotozoal, antigiardial, antibacterial toward Helicobacter pylory, convenient in treatment of burns and infectious wounds, edema, eczema, urticarial, the cold and scolices of Hydatid cysts [9-11]. Significant antioxidant activities have been reported from its flowers [12]. Recently, good antiemetic and neuroprotective, antidepressant and anti-inflammatory activities of this plant have been reported from our team [13-15]. Effect of Indole-3-acetic acid (IAA) and Naphthalene acetic acid (NAA) growth hormones on the chemical composition of volatile oils of S. ebulus leaf have been reported recently [16]. In this study, the effect of growth hormones (GA3) on the chemical composition of volatile oil was evaluated. It seems/ appears that some compounds in the leaf composition can play a role in the interpretation of the mode of action in some biological and/or toxic activities that have been reported previously from S. ebulus [8].

Methods

Chemicals

Gibberellic acid (GA3) was purchased from Merck (Germany). The structure of phytohormone has been shown in Figure 1. and Treatments

S. ebulus plant was selected in the same condition (temperature, light, humidity) in the suburb of Sari (Iran) in August 2009 and identified by Dr B. Eslami (Department of Biology, Islamic Azad University of Qhaemshahr, Iran). A voucher specimen (no.382) was deposited at the Herbarium of the Sari School of Pharmacy. The experimental work was divided into 2 parts. The leaves of *S. ebulus* were sprayed three times a week by distilled water (as control) and solution of GA3 (100 ppb). Following the treatment, at the end of the week, the leaves were collected from each of the samples specifically and were dried in the dark and a non humid environment. The conditions under which the treatments were carried out were the same during the whole experiment.

Extraction of the essential oil

70 g of dried leaves were separately subjected to hydrodistillation, using a Clevenger-type apparatus for 3 h. The oils obtained were dried over anhydrous sodium sulfate and stored in a sealed vial at a low temperature before analysis (0.1%). The oils were analyzed using GC and GC/MS analysis.

Gas chromatography (GC)

The GC analysis was carried out using a Hewlett Packard 6890N GC System equipped with a FID detector. 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°C at a rate of 5 °C/min, and kept constant at 220 °C for 20 min. The injector and detector temperature was 270 °C. The injection volume was 1 µL. Helium and was used as a carrier gas at a flow rate of 1 ml/min. The quantitative data were obtained electronically from FID area percent without the use of correction factors.

Gas chromatography-mass spectrometry (GC-MS)

GC–MS analysis was performed using a Hewlett Packard 5973N mass selective detector connected with/to an HP 6890N gas chromatograph. The same capillary GC conditions as described above. MS was taken at 70 eV.

Identification of constituents

The constituents were identified by comparing their mass spectra with those of authentic compounds or with reference spectra in the computer library

(AUTOINT 1. E), and confirmed by comparing the retention indices with those of authentic compounds, or with data in the literature [17].

Results and Discussion

Table I lists the oil constituents identified in the leaves and of S. ebulus, the relative GC peak areas of these constituents, and their experimental retention indices on the HP-5 MS column. Sixty components have been identified. In qualitative and quantitative analysis of leaf oils, the following results were obtained. Longifolene is a naturally occurring sesquiterpene, because of its significance in the fragrance industry, its role in a number of rearrangement reactions has been intensively investigated [18]. Longifolene is eradicated completely by treating with GA3. But this hormone increased isolongifolene at the concentration of 100 ppb. Borneol is a bi-cyclic monoterpene which is synthesized from turpentine oil or camphor, and is present in the essential oils of numerous medicinal plants, including sage, valerian, chamomile, rosemary and lavender. Borneol is a common ingredient in many traditional Chinese herbal formulas with current Chinese name bingpian. It is used more frequently for topical applications than for internal use. These applications are numerous, but especially apply to injuries, rheumatic pains, haemorrhoids, burns, skin diseases, and ulcerations of the mouth and ear. It increases production of gastric juices; tones the heart and improves circulation; treats bronchitis, coughs and colds; reduces swelling; relieves stress; and can be used as a tonic to promote relaxation and reduce exhaustion [19]. It is eradicated completely by treating with GA3. Elemene is a mixture of sesquiterpene compounds extracted from ginger plants curcuma, with outstanding advantages of a broad anti-tumor spectrum, curative effect, and less adverse reaction [20]. There are three major components of β, γ, δ isomers. Recently elemene emulsion has been used widely in clinical treatment for malignant effusions, lung cancer, gastrointestinal cancer, brain cancer, gynecological cancer, breast cancer, skin cancer, and other tumors [21]. Elemene is a volatile oil with poor oral absorption and low bioavailability, limiting its clinical applications [22]. Both γ and δ elemene has been eradicated in plant treated GA3.

Aromadendrene is the main constituent of the distillation tail1 of the essential oil of Eucalyptus globulus [23, 24]. Aromadendrene is a cheap and abundantly available chiral starting material for organic syntheses. It has been shown that many other useful intermediates and natural products can be obtained from this compound [23]. Aromadendrene was eradicated completely by treating with GA3. β-Selinene is the major sesquiterpene of calamondin fruits [25]. β-Selinene, also, was eradicated completely by treating with GA3. trans-caryophyllene is a sesquiterpene with pronounced anti-inflammatory properties [26]. GA3 eradicated it completely. Also, β- caryophyllene oxide was eradicated in treating with GA3.

Eugenol can inhibit prostaglandins synthesis [27]. There have been many studies on the cytotoxicity of o-methoxyphenols such as eugenol and isoeugenol [28]. These molecules possess prooxidant as well as antioxidant activities 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 [28]. Eugenol was eradicated completely by treating with GA3 but isoeugenol, which has never been reported before in control group, was produced noticeably in treating with GA3 hormone Germacrene D is a sesquiterpene hydrocarbon [29].

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 behaviour have been documented in the literature [30]. Germacrene D was eradicated completely by treating with GA3 but GA3 increased Germacrene B content about 28 times. 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 [29]. β-Bisabolene was eradicated completely by treating with GA3 but Bisabolene -11-ol which has never been reported before in control group, was produced noticeably in treating with GA3 hormone. α-Thujone is the toxic monoterpenoid in some herbal medicines and is reported to have acute toxic effect and causes convulsions [31]. Long-term ingestion of plants containing this compound can cause hallucinations, sleeplessness, tremor, convulsions, and paralysis, a syndrome called absinthism. Animal experiments have shown that α-thujone is neurotoxic [31]. The presence of α-Thujone in leaf composition can improve some plant's toxicity we have reported recently [8]. α and β-Thujone were eradicated completely by treating with GA3. β-Caryophyllene is a bicyclic sesquiterpene (that is quite widely

distributed in plants. It possesses anticarcinogenic activity [32] and could play a role in plant defense [33]. 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. Caryophyllene was detected in 33% of 300 analyzed cosmetic products on the Dutch market in the beginning of the 1990s [34]. β-Caryophyllene is known for its anti-inflammatory and local anaesthetic activities [35]. β-Caryophyllene was eradicated completely by treating with GA3. Several compounds i.e. No. 1,2, 3, 6, 7, 9, 18, 21, 22, 30, 41, 42, 52, 56 were eradicated completely by treating with GA3 hormone. The remaining items in Table I, No.61-69, have been produced noticeably in treating with GA3 hormone which have never been reported before in control group. There are no reported biological activities from these compounds in the literature.

In conclusion, these results indicate that the phytohormone GA3, used in this study has significant effect on the concentration of essential oil components in S. ebulus. Some components were increased and some of them decrease significantly. In some cases, the compounds were eradicated or induced completely. It seems a useful method for changing the concentration of the essential oil compounds.

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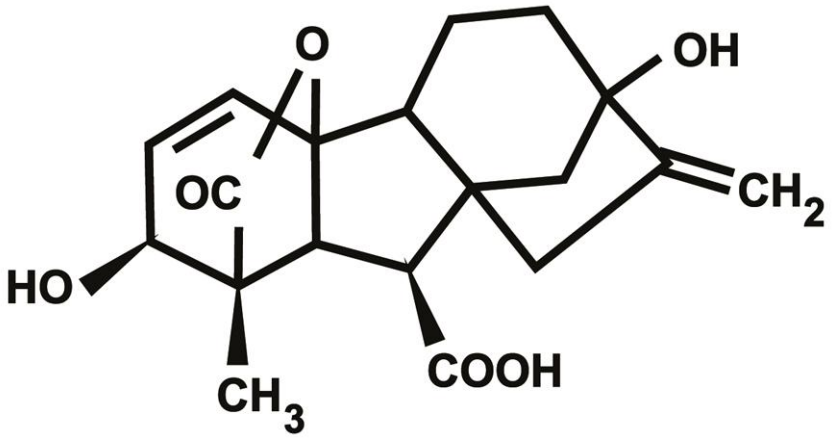

Figure 1. Structure of Gibberellic acid (GA3)

Table1. Chemical composition of the essential oils of S. ebulus leaf (Control group) and Gibberellic acid (GA3) treated (Hormones treated).

No.	Compound	KI	Blank %	GA3 %
1	β-pinene	976	0.57	
2	α-terpinene	1012	0.28	
3	linalool oxide	1062	0.74	
4	α-thujone	1094	1.19	
5	β-thujone	1101	0.81	
6	ocimene oxide	1110	1.16	
7	ocimenone	1124	0.38	
8	Camphor	1129	0.76	
9	iso pulegol	1135	0.09	
10	pino carvone	1140	0.23	
11	iso borneol	1147	0.92	
12	Borneol	1156	0.58	
13	terpinen-4-ol	1167	2.39	
14	Myrtenal	1174	0.33	
15	α-terpineol	1178	1.45	
16	Myrtenol	1184	0.64	
17	verbenone	1191	1.19	
18	Fragranol	1196	0.44	
19	trans carveol	1200	0.27	
20	cis carveol	1211	3.86	
21	Pulegol	1215	0.11	
22	Carvone	1218	0.5	
23	Pulegone	1220	0.98	
24	Chavicol	1229	2.01	
25	Geraniol	1236	1.52	0.96
26	Geranial	1245	0.95	
27	isoestragol	1260	1.39	
28	Safrol	1269	0.19	1.31
29	bornyl ac	1272	0.92	
30	Carvacrol	1281	0.72	
31	Piperitone	1286	1.24	4.07
32	cis-pinocarvyl ac	1293	1.24	
33	myrtenyl ac	1300	1.05	
34	trans-verbenol ac	1308	3.74	1.54
35	trans carvyl ac	1318	2.32	
36	Eugenol	1332	2.05	
37	δ-elemene	1339	2.32	
38	α-cubebene	1358	5.22	
39	geranyl ac	1363	5.65	
40	α-bourbonene	1376	3.85	
41	α-copaene	1379	1.88	
42	β-cubebene	1388	0.29	
43	iso-longifolene	1392	1.93	2.25
44	Cyperene	1404	3.13	
45	longifolene	1408	0.28	
46	β-gurjunene	1412	0.84	
47	β-caryophyllene	1418	2.14	
48	β-caryophyllen oxide	1425	3.28	
49	γ-elemene	1434	0.99	
50	aromadendrene	1440	1.77	
51	dehydroaromadendrane	1458	0.72	4.07
52	Germacrene D	1480	6.89	
53	β-selinene	1483	0.66	
54	epi- cubebol	1491	0.25	
55	β-bisabolene	1507	11.4	
56	Cubebol	1513	0.81	
57	δ-cadinene	1521	1.66	
58	Germacrene B	1557	0.18	4.97
59	caryophyllen epoxide	1565	0.51	
60	caryophyllenol I	1650	1.45	9.48
61	Selinen-γ	1527		25.29
62	Copa borneol	1605		6.63
63	α-cadinol	1639		3.68
64	caryophyllenol II	1661		4.77
65	bisabolene oxide	1553		3.52
66	β-elemol	1540		4.48
67	iso-eugenol-E	1422		2.68