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Original Research Article

Effect of Phytohormones on the Composition of *Sambucus ebulus* Leaf Essential Oil

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Abstract

Purpose: *To evaluate the effect of growth hormones - naphthalene acetic acid (NAA) and indole-3-acetic acid (IAA) - on the essential oil of Sambucus ebulus leaf.*
Methods: *The leaves of S. ebulus were sprayed three times in one week with distilled water (as control) or with a solution of either NAA or IAA (150 ppb). Following the treatment, the leaves were collected from each of the plant and dried in the dark in a dry environment. The essential oil content of the leaves was obtained by hydrodistillation and analyzed by gas chromatography (GC) and GC-mass spectrometry (GC-MS)*
Results: *Sixty constituents were identified in the plant oil, some of which could have been responsible for the plant's biological and/or toxicological activities. The results indicate that NAA and IAA exerted significant effect on the composition of the essential oil, increasing some components and decreasing some others significantly. In some cases, certain compounds were eliminated completely from the oil.*
Conclusion: *The use of phytohormones seems a useful strategy for modifying the composition of the essential oil in plants.*

Keywords: *Essential oil; Indole-3-acetic acid; Phytohormones, 1-Naphthaleneacetic acid; Sambucus ebulus.*

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INTRODUCTION

Four species of the genus *Sambucus* (Caprifoliaceous) grow in Iran. Of these species, *S. ebulus* extensively grows in the northern regions of Iran [1]. Iranian traditional medicine uses the leaves and rhizomes of *S. ebulus* in treating some inflammatory problems such as, bee and nettle bites, arthritis, and a sore-throat [2]. 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 common cold and rheumatism [3]. Recently good antioxidant activities were reported [4].

Phytohormones play an important role in the regulation of germination, growth, reproduction, and protective responses of plants against stress. Mass spectrometry (MS) is the most powerful tool for the determination of phytohormones due to its high sensitivity and selectivity. The use of GC-MS for this purpose has also been reported [5].

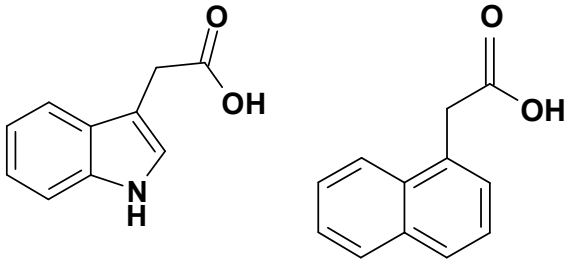
Indole-3-acetic acid (IAA) is the major plant growth hormone and is involved in the regulation of almost every step of plant development [6]. Naphthalene acetic acid (NAA) is widely employed in agriculture as a plant growth regulator. NAA prevents premature flowering, fruit drop and controls re-growth of tree sprouts after trimming.

In this study, the effect of growth hormones (NAA and IAA) on the chemical composition of volatile oils were evaluated because it seems that some compounds in the leaf may play a role in some biological and/or toxicological activities that have been reported previously for *S. ebulus*.

EXPERIMENTAL

Chemicals

IAA and NAA were purchased from Merck (Germany). The structures of the two phytohormones are shown in Figure 1.



Indole-3-acetic acid (IAA)

Naphthalene acetic acid (NAA)

Figure 1: Structures of NAA and IAA.

Plant materials and treatments

S. ebulus plant was collected from the suburb of Sari, Mazandaran Province, north of 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 3 parts. The leaves of *S. ebulus* were sprayed three times in one week with distilled water (control) or a solution of either NAA or IAA (150 ppb). Following the treatment, the leaves were collected from each of the plants and dried in the dark and a non-humid environment. The conditions under which the treatments were carried out were the same for the duration of the experiment. All experiments were repeated thrice.

Isolation of the Essential Oil

The air-dried leaves were subjected to hydrodistillation, using a Clevenger-type apparatus for 4 h. The oil obtained was dried over anhydrous sodium sulfate and stored in a sealed vial at a low temperature before analysis. The oils were analyzed using GC and GC/MS analysis.

Gas chromatography (GC)

Gas chromatographic analysis was carried out on a Hewlett Packard 6890N GC system with FID detector and a HP-5 MS (30 m × 0.320 mm) capillary column. 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 was used as a carrier gas at a flow rate of 1 ml/min.

Gas chromatography-mass spectrometry (GC-MS)

GC–MS analysis was performed using a Hewlett-Packard 5973N mass selective detector connected to an HP 6890N gas chromatograph. The same capillary GC conditions as described above were used. MS measurement was carried out at 70 eV.

Identification of constituents

The components of the oil were identified by their retention times, retention indices relative to C_9-C_{28} *n*-alkanes, computer matching with AUTOINT 1. E library and comparison of their mass spectra with those of authentic samples or with data already available in the literature [7]. The composition of the identified compounds was computed from the GC peak area without any correction factor and was calculated relatively.

RESULTS

Hydrodistillation of the dried leaves of *S. ebulus* resulted in a light yellowish oil with a yield of 0.1% v/w. As shown in Table 1(a), (b) and (c), sixty components were identified in the oil, representing 97.31% of its total content. Longifolen was eradicated from the plant leaves treated with IAA when compared with control. On the other hand, NAA increased its content about 5 times. Both NAA and IAA substantially increased γ- elemene in plant oil. δ- Elemene

Table 1(a): Chemical composition of the essential oils of *S. ebulus* leaf (control group), naphthalene acetic acid (NAA) - and indole-3-acetic acid (IAA)-treated leaf treated)

No.	Component	KI	*S. ebulus*	% IAA	% NAA
			Area (%)	*150 ppb*	*150 ppb*
1	β-Pinene	976	0.57		
2	α-Terpinene	1012	0.28		
3	Linalool oxide	1062	0.74	0.64	
4	α-Thujone	1094	1.19	0.94	
5	β-Thujone	1101	0.81	0.95	
6	Ocimene oxide	1110	1.16	1.38	
7	Ocimenone	1124	0.38		
8	Camphor	1129	0.76	1.45	
9	Iso pulegol	1135	0.09		
10	Pino carvone	1140	0.23		
11	Iso borneol	1147	0.92	1.21	
12	Borneol	1156	0.58		
13	Terpinen-4-ol	1167	2.39	2.12	
14	Myrtenal	1174	0.33		
15	α-Terpineol	1178	1.45	2.12	
16	Myrtenol	1184	0.64		
17	Verbenone	1191	1.19	1.19	
18	Fragranol	1196	0.44		
19	Trans carveol	1200	0.27		
20	Cis carveol	1211	3.86	5.06	
21	Pulegol	1215	0.11		
22	Carvone	1218	0.5		
23	Pulegone	1220	0.98	0.88	
24	Chavicol	1229	2.01	2.37	
25	Geraniol	1236	1.52	0.69	1.26
26	Geranial	1245	0.95	1.49	0.7
27	Iso estragol	1260	1.39		
28	Safrol	1269	0.19		0.59

KI = Kovats Index on HP-5 column

Table 1(b): Chemical composition of the essential oils of *S. ebulus* leaf (control group), Naphthalene acetic acid (NAA) - and indole-3-acetic acid (IAA)-treated leaf

No.	Component	KI	*S. ebulus*	% IAA	% NAA
			Area (%)	*150 ppb*	*150 ppb*
29	Bornyl ac	1272	0.92		
30	Carvacrol	1281	0.72		
31	Piperitone	1286	1.24		2.07
32	Cis-pinocarvyl ac	1293	1.24		
33	Myrtenyl ac	1300	1.05		0.51
34	Trans-verbenol ac	1308	3.74	1.85	0.66
35	Trans carvyl ac	1318	2.32		1.87
36	Eugenol	1332	2.05	1.76	
37	δ-Elemene	1339	2.32	0.98	
38	α-Cubebene	1358	5.22	6.69	
39	Geranyl ac	1363	5.65	5.73	
40	α-Bourbonene	1376	3.85	4.85	
41	α-Copaene	1379	1.88		
42	β-Cubebene	1388	0.29		
43	Iso-longifolene	1392	1.93	1.36	
44	Cyperene	1404	3.13	3.51	
45	Longifolene	1408	0.28		1.35
46	β-Gurjunene	1412	0.84		
47	β-Caryophyllene	1418	2.14	2.93	
48	β-Caryophyllen oxide	1425	3.28	1.96	
49	γ-Elemene	1434	0.99	3.88	4.23
50	Aromadendrene	1440	1.77	12.09	
51	Dehydro aromadendrane	1458	0.72		4.78
52	Germacrene D	1480	6.89		
53	β-Selinene	1483	0.66	7.79	
54	Epi- cubebol	1491	0.25		1.38
55	β-Bisabolene	1507	11.4		4.64
56	Cubebol	1513	0.81		

KI = Kovats index on HP-5 column

Table 1(c): Chemical composition of the essential oils of *S. ebulus* leaf (control group), Naphthalene acetic acid (NAA) - and indole-3-acetic acid (IAA)-treated

No.	Component	KI	*S. ebulus*	% IAA	% NAA
			Area (%)	*150 ppb*	*150 ppb*
57	δ-Cadinene	1521	1.66	0.67	
58	Germacrene B	1557	0.18		1.28
59	Caryophyllen epoxide	1565	0.51		
60	Caryophyllenol I	1650	1.45	0.91	8.38
61	β-Bourbonene	1386		1.27	
62	α-Caryophyllen oxide	1416		0.87	
63	α-Humelene	1453		5.41	1.85
64	γ-Muurolene	1473			1.13
65	Selinen-y	1527			17.07
66	Copa borneol	1605			3.72
67	Iso spathulenol	1628			8.73
68	α-Cadinol	1639			0.89
69	Caryophyllenol II	1661			4.77
70	Farnesal	1670			1.47
		Total	97.31		

KI = Kovats index on HP-5 column

was eradicated from the leaves treated with NAA but decreased by IAA (2.32 for control *vs* 0.98 for treated). Aromadendrene was increased 7 times in content by treating with IAA, compared with control while NAA eradicated it completely. Dehydroaromadendrene was dramatically increased by NAA (0.72 *vs* 4.78 for control and test, respectively); on the other hand, IAA eradicated it completely. β-selinene elevated 12 times by IAA treatment while NAA eradicated it completely in treated plants.

α-Humulene was not detected in control group but both NAA and IAA significantly induced production of α-Humulene in treated plants. IAA increased *trans*-caryophyllene conyents but NAA eradicated it completely. β- caryophyllene oxide was decreased by INN and eradicated it in those treated with NAA. As a result of treatment, cis pinocarvyl acetate was generated by NAA and INN, unlike in control. IAA reduced α-thujone (1.19 *vs* 0.94 for control and treated, respectively) but NAA eradicated it completely. Several compounds, i.e., nos. 7, 9, 10, 12, 14, 16, 18, 19, 21, 22, 27, 29, 30, 41, 42, 46, 52, 56 were eradicated completely when the leaves were treated with NAA and IAA hormones. The other compounds in Table 1 (c), i.e., nos. 64 - 70, were produced following treatment with NAA. These compounds have previously not reported for the plant.

DISCUSSION

Sixty components were identified in the oil. Longifolene is a naturally occurring sesquiterpene whose role in a number of oxidation and rearrangement reactions, because of its significance in the fragrance industry, has been intensively investigated [8]. It was eradicated in the plant treated IAA in comparison with blank. NAA increased it about 5 times. 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 [9]. There are three major components of β, γ, δ isomers. Recently, elemene emulsion has been used widely in clinical treatment for many malignancies and tumors [10].

Both NAA and IAA significantly increased γ-Elemene in the plant oil. δ- Elemene has been

eradicated in plant-treated NAA but decreased by IAA. Aromadendrene is the main constituent of the distillation tail of the essential oil of Eucalyptus globulus. 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 [11]. Aromadendrene was increased 7 times in content by treating with IAA. NAA eradicated it completely. β-Selinene is the major sesquiterpene of calamondin fruits. It was increased 12 times by treating with IAA. NAA eradicated it completely. α-Humulene and *trans*-caryophyllene are plant sesquiterpenes with pronounced anti-inflammatory properties [12].

α-Humulene exhibits marked antiallergic and anti-inflammatory properties [13]. It was not detected in control groups but both NAA and IAA significantly induced its production in treated plants. α-Thujone is the toxic monoterpenoid in some herbal medicines and is reported to have acute toxic effect and causes convulsions [14]. 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 [14]. The presence of α-Thujone in the leaf can increase the plant's toxicity as was reported recently [1].

CONCLUSION

It seems that treatment of plants with some growth hormones (NAA and IAA) would be a useful method for modifying the chemical composition of *S. ebulus* leaf essential oil. NAA and IAA significantly influence the concentration and composition of the essential oil of *S. ebulus*.

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