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COMPARISON OF HORMONAL CHANGES (NAA,IAA,GA_3) OF THE COMPOSITION OF ESSENTIAL OIL FROM SAMBUCUS EBULUS LEAF

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Abstract

Plant hormones are a group of chemically diverse molecules that control virtually all aspects of plant development. Classical plant hormones were identified many decades ago in physiology studies that addressed plant growth regulation. In this study the effects of growth hormones (NAA, GA_3 ,IAA) on the components of the essential oil of *Sambucus ebulus* leaf of(*Caprifoliaceous*) was evaluated. Water distillation method was used for extracting essential oil by Clevenger apparatus and then were analysed by capillary GC and GC/MS. Eighty seven constituents were identified in the leaf oil. Some detected compounds can be responsible for plant biological and toxic activities.

Keywords: *Sambucus Ebulus; essential oil; 1-naphthaleneacetic acid ;gibberellic acid ; indole-3-acetic acid .*

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] (Fig1). Iranian traditional medicine uses, in various occasions, 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]. 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 [1,4]. Recently we have reported good antioxidant activity of SE fruit and flowers [5]. Studies have established extracts of some plants which are rich in flavonoids [6] and many of these phenolic compounds have shown to be cytoprotective by reducing oxidative stress [7], thereby giving a solid basis to the proposal that the antioxidant content of plants could account for its cardioprotective properties [8]. Plant hormones (phytohormones) are small organic molecules, commonly used to increase grain production [9] 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 [10]. Phytohormones are usually classified into four groups: auxines, gibberellines, cytokinines and inhibitors [11]. Gibberellic acid (GA_3), indole-3-acetic acid (IAA) and abscisic acid (ABA) are the chief representatives of gibberellins, auxines and inhibitors, respectively.

The quantitative analysis of the three phytohormones is often required in agriculture and plant physiology. However, many researches until now have focused on a specific phytohormone or certain group of phytohormones but ignored the others. Common purification procedures such as liquid-phase extraction (LPE), solid-phase extraction (SPE) [12,16], vapor-phase extraction [17] and solid-phase microextraction have been employed for the purification of phytohormones in plants. However, LPE is extremely expensive and adverse to the environment and human health due to the use of large amounts of organic solvents. Several methods have been used for the analysis of phytohormones. Among them, high performance liquid chromatography (HPLC) coupled to ultraviolet (UV) [13] or fluorescence[18] or coulometric detection [19] has been described and immunoassay [20] has also been employed. Mass spectrometry (MS) is the most powerful detector for the determination of phytohormones due to its high sensitivity and selectivity. Gas chromatography coupled to mass spectrometry (GC–MS) [14] was also reported. gibberellic acid (GA_3), accelerates and improves the yield of a wide variety of plants by increasing cell division [9,21]. Although GA_3 is widely used in agriculture, its effects on human health are not well-explored. Only a few experiments examined possible toxic effects of plant growth regulators, including GA_3, in mammals. Indole-3-acetic acid (IAA) is the major plant growth hormone and is involved in the regulation of almost every step in plant development.The routes by which plants synthesize IAA are only incompletely understood at present, but several lines of evidence, including the impossibility to select IAA-deficient mutants, suggest that more than a single pathway is operative[22].Naphthalene acetic acid (NAA) is widely employed in agriculture as a plant growth regulator (a phytohormone or synthetic "auxin", from Greek auxein which means to grow). Among other effects, NAA prevents premature flowering, fruit drop and it controls regrowth of tree sprouts after trimming. The use of essential oils can have significant effects on both clinical and experimental pain. For example, cancer pain and the associated anxiety are alleviated by exposure to lavender aroma [23]. In this study, the effects of growth hormones (NAA, GA_3 ,IAA) on 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

Solvents and Chemicals

Solvents and fine chemicals were purchased from Merck or Fluka Companies and were of highest purity and analytical grade. GA_3, IAA and NAA were purchased from Merck(Germany). The structures of the three phytohormones are shown in Fig2.

Plant Materials and treatments

In the present study plant of *Sambucus ebulus* in the same condition (temperature,light,humid) in suburb sari in August 2009 in order to experimental work divided to 10 part.Treatments were (1) control,(2) solution of GA_3 (100,50,25ppb);(3) solutions of NAA,IAA(150,100,50).leaves of *Sambucus ebulus* in each of these pieces were sprayed three time in week in treatment solution with specified volume with different concentrations of GA_3 (100,50,25 ppb) ;IAA

,NAA(150,100,50 ppb) and in distilled water only as a control.After treatment the end of week leaves of *Sambucus ebulus* were collected from each of pieces specifically and dried in dark and avoid humid .Treatments conditions (temperature, relative humidity and photoperiod) were the same during the whole experiment.

Preparation of plant samples and essential oil extraction

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

Gas chromatography (GC)

GC analysis was carried out using a Hewlett Packard - Agilent - Model 6890 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. Injector and detector temperature was 270°C. Injection volume was 1 µL.Helium was used as 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 Pckard - Model 5973 mass selective detector connected with 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 comparison of their mass spectra with those of authentic compounds or with reference spectra in the computer library (AUTOINT 1. E), and confirmed by comparison of retention indices with those of authentic compounds, or with data in the literature .

Results

Composition of the essential oils

Table 1 lists the oil constituents identified in the leaves and of *Sambucus ebulus*, the relative GC peak areas of these constituents, and their experimental retention indices on the HP-5 MS column. Eighty-seven components have been identified.

Conclusions

In qualitative and quantitative analysis of leaf oils from *Sambucus ebulus* by GC and GC/MS according to table 1 of the following results were obtained.

1. cis pinocarvyl ac in effect of treatment hormone GA_3 (25 ppb), compared to the control is about 7 turns and the effect of treatment hormone NAA (100 ppb) than the control 3.5 turns.
2.Longifolene in effect of treatment hormone (IAA 50ppb) than the control 9 turns and has been treated GA_3 has been deleted.
3. γ-elemene have been used in treating lung cancer and as pain decrease used to be, in effect of treatment hormone (IAA 100 ppb) than the control 12.5 turns.
4. Aromadendrene in effect of treatment hormone IAA about 12 turns over the control increased.
5. Dehydro aromadendrene in effect of treatment hormone NAA to control the intensity has increased.
6. β-Selinene in effect of treatment hormone IAA, 8 turns increased compared to control.
7. Germacren B in effect of treatment hormone GA_3, 50 turns over the control increased.
8. Caryophyllene epoxide in effect of treatment hormone GA_3, 60 turns and in effect of treatment hormone NAA (100 ppb), 50 turns more than the control increased.
9.Caryophyllenol I in effect of treatment hormone NAA and GA_3,8 to12times increased campared to control.
10. rest of the compounds in Table 1 from No. 61 to 87 in effect of treatment hormone NAA and GA_3 were significantly produced in the control did not exist previously.

This results indicates that growth hormones used in this study have increased and reduced effects on the concentration of essential oil components in the plant *sambucus ebulus* and is a useful method for changing of the concentration of the essential oil compounds with these hormones.

Longifolene is a naturally occurring sesquiterpene whose role in a number of oxidation and rearrangement reactions has been intensively investigated [24] due to its significance to the fragrance industry. 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 [25]. Among its three major components of β, γ, δ isomers, β-elemene is the main active ingredient [26] and thus used commonly for content determination of elemene. Elemene is a volatile oil with poor oral absorption and low bioavailability , [27] limiting its clinical applications.

Aromadendrene is a cheap and abundantly available chiral starting material for organic syntheses. It has been shown by our laboratory that many other useful

intermediates and natural products can be obtained from aromadendrene [28-29].

β-Selinene is the major sesquiterpene of calamondin fruits and the protein responsible for its synthesis from farnesyl diphosphate has been purified 83-fold. Cedrol is a crystalline natural substance derived from cedar wood (Juniperus Virginia) oil. Cedrol or cedar wood oil, which diffuses a week aroma, has been commonly used as an ingredient of shampoo, washing soap, facial soap, essence, etc. to enhance other fragrances. Farnesol is a member of a class of compounds known as non-sterol isoprenoids [30].

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Table 1 . Comparison chemical composition of the essential oil of *S. ebulus* leaf.

S/R	Compound	KI	Blank	% IAA			% NAA			% GA3		
			%	150 ppb	100 ppb	50 ppb	150 ppb	100 ppb	50 ppb	100 ppb	50 ppb	25 ppb
1	β-pinene	976	0.57			1.09						
2	α-terpinene	1012	0.28									
3	linalool oxide	1062	0.74	0.64		0.78						
4	α-thujone	1094	1.19	0.94	1.19	1.14						
5	β-thujone	1101	0.81	0.95	1.18	1.25						
6	ocimene oxide	1110	1.16	1.38	1.41	2.36						
7	ocimenone	1124	0.38									
8	camphor	1129	0.76	1.45	1.55	1.53						
9	iso pulegol	1135	0.09									
10	pino carvone	1140	0.23								1.89	
11	iso borneol	1147	0.92	1.21		1.42						
12	borneol	1156	0.58			0.58						
13	terpinen-4-ol	1167	2.39	2.12	2.39	2.76						
14	myrtenal	1174	0.33			0.72						
15	α-terpineol	1178	1.45	2.12	0.93	1						
16	myrtenol	1184	0.64			1.99						
17	verbenone	1191	1.19	1.19	2.03	1.54						
18	fragranol	1196	0.44									
19	trans carveol	1200	0.27									2.21
20	cis carveol	1211	3.86	5.06	4.79				0.35			
21	pulegol	1215	0.11			5.1						
22	carvone	1218	0.5									
23	pulegone	1220	0.98	0.88		1.38						
24	chavicol	1229	2.01	2.37	1.51	2.81						
25	geraniol	1236	1.52	0.69		0.7	1.26			0.96		
26	geranial	1245	0.95	1.49		2.45	0.7		4.46			5.0
27	iso estragol	1260	1.39			1.03						
28	safrol	1269	0.19				0.59		1.31	1.31		
29	bornyl ac	1272	0.92			0.52						2.6
30	carvacrol	1281	0.72									
31	piperitone	1286	1.24			1.01	2.07			4.07		

32	cis-pinocarvyl ac	1293	1.24			0.64		4.21				8.42
33	myrtenyl ac	1300	1.05				0.51		2.43			
34	trans-verbenol ac	1308	3.74	1.85	2.38	3.91	0.66		0.37	1.54		
35	trans carvyl ac	1318	2.32				1.87		0.97			3.5
36	eugenol	1332	2.05	1.76	3.21	1.84						
37	δ-elemene	1339	2.32	0.98	1.02	2.68						
38	α-cubebene	1358	5.22	6.69	7.62	9.16			2.37			
39	geranyl ac	1363	5.65	5.73	8.22							
40	α-bourbonene	1376	3.85	4.85	4.13	4.2						
41	α-copaene	1379	1.88		1.78							
42	β-cubebene	1388	0.29									
43	iso-longifolene	1392	1.93	1.36	3.42	2.32				2.25		
44	cyperene	1404	3.13	3.51	4.37							
45	longifolene	1408	0.28			3.06	1.35		0.79			
46	β-gurjunene	1412	0.84		1.65							
47	β-caryophyllene	1418	2.14	2.93	5.06	2.35			0.85			
48	β-caryophyllen oxide	1425	3.28	1.96	7.97	0.87						
49	γ-elemene	1434	0.99	3.88	12.7	2.36	4.23		0.7			
50	aromadendrene	1440	1.77	12.09		6.32			3.37			
51	dehydro aromadendrane	1458	0.72			5.18	4.78	4.67	21.7	4.07		4.12
52	Germacrene D	1480	6.89									
53	β-selinene	1483	0.66	7.79		6.13			0.82			
54	epi- cubebol	1491	0.25				1.38					
55	β-bisabolene	1507	11.4		11		4.64		1.33			
56	cubebol	1513	0.81									
57	δ-cadinene	1521	1.66	0.67	0.77				1.28			
58	Germacrene B	1557	0.18				1.28	7.53		4.97	9.65	4.79
59	caryophyllen epoxide	1565	0.51					24.72			18.07	29.3
60	caryophyllenol I	1650	1.45	0.91	2.05		8.38	10.41	2.64	9.48	11.92	15.3
61	β-bourbonene	1386		1.27		2.22						
62	α-caryophyllen oxide	1416		0.87								
63	α-humelene	1453		5.41		1.64	1.85					
64	pulegone epoxide	1233				0.44						
65	α-bisabolol	1665				1.99						
66	γ-muurolene	1473					1.13					

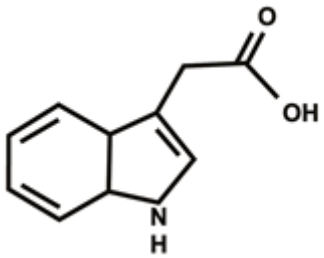
67	Selinen-y	1527					17.07	4.29	2.33	25.29		3.04
68	Copa borneol	1605					3.72			6.63		
69	iso spathulenol	1628					8.73					
70	α-cadinol	1639					0.89			3.68	3.22	
71	caryophyllenol II	1661					4.77	1.64		4.77		
72	farnesal	1670					1.47					
73	bisabolene oxide	1553						10.03		3.52	2.66	7
74	globulol	1583						19.99			16.97	
75	Valeranone	1664						5.38			6.54	2.06
76	iso bornyl ac	1277							0.53			
77	β-elemol	1540							2.23	4.48		3.54
78	iso-longifolan-7-α-ol	1610							36.4			
79	farnesol	1680							11.5			
80	bisabole-12-ol	1759							18.2			
81	iso-eugenol-E	1422								2.68		
82	bisabolene-11-ol	1613								2.35		
83	Cubenol -1 epi	1631								17.93		3.39
84	cedrol	1569									18.53	
85	iso longifolol	1729									4.92	
86	albicanol	1735									5.61	
87	β-bisabolol	1658										5.55

A

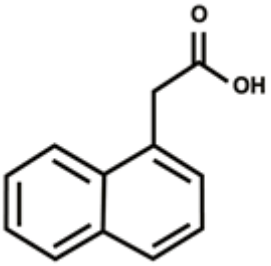
B

C

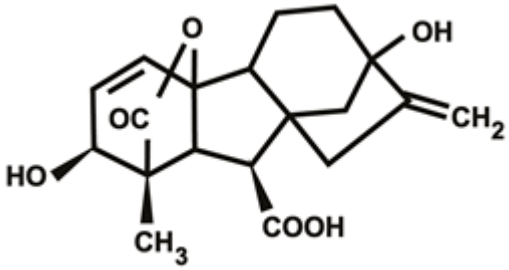
Fig1. *Sambucus ebulus*; A: Friut, B:leaf, C:Flower



Indole-3-acetic acid (IAA)



Naphthalene acetic acid (NAA)



Gibberellic acid (GA3)

Fig. 2. Structures of the NAA,GA_3,IAA.