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.

INTRODUCTION

Four species of the genus Sambucus (Caprifoliaceae) 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.

![Figure 1: Structures of NAA and IAA.](image)

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 C₉-C₂₈ n-alkanes, computer matching with AUTJOINT 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-Octen-1-ol
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 Area (%) | % IAA  | % NAA  |
|-----|----------------|-----|--------------------|--------|--------|
|     |                |     |                    | 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   | Ocimenone      | 1110| 1.16               | 1.38   |        |
| 7   | Ocimene oxide  | 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* Area (%) | % IAA 150 ppb | % NAA 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 aromadendrene          | 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 Area (%) | % IAA 150 ppb | % NAA 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 contents 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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