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SHORT COMMUNICATION

# Essential Oil Constituents of *Angelica glauca* Edgew. Roots: An Endangered Species from Uttarakhand Himalaya (India)

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**Abstract** The roots of *Angelica glauca* Edgew., a high value critically endangered plant, were collected from two alpine Himalayan locations of Uttarakhand (India). The essential oils were obtained by hydro-distillation, analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) in order to determine the variation of concentration in their constituents. A total of 26 compounds were identified in both the oils, representing 97.7–98.2 % of total oil compositions. The major components of *A. glauca* oils were characterised as (Z)-ligustilide (40.6–53.0 %), (Z)-butylidene phthalide (20.7–32.8 %), (E)-butylidene phthalide (2.5–5.9 %) and (E)-ligustilide (2.1–2.3 %). Due to the higher relative area quantum of monomeric lactones (phthalides and ligustilides) in *A. glauca* populations (69.3–90.6 %) growing in Uttarakhand, there is a need to develop propagation protocol for mass multiplication and in situ and ex situ conservation.

**Keywords** *Angelica glauca* · Apiaceae · Essential oil · (Z)-ligustilide · Himalaya

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Gandhrayan (*Angelica glauca* Edgew.; Family Apiaceae), a high value medicinal and aromatic plant is native to the Himalaya and distributed at the altitudes of 2,000–3,800 m in North-Western Himalaya [1]. Due to the high demand of raw material, unsustainable harvesting, over exploitation and grazing problems, naturally growing populations of the species have become very rare and restricted to a very few habitats [2], hence the species is now reported to be endangered for the Himalayan region, adopting IUCN criteria [3]. The whole herb is reported to be useful as stimulant, cordial, appetizer, dyspepsia, cardioactive, carminative, expectorant, diaphoretic and also in stomach troubles and urinary disorders, constipation and gastric problems, bilious complaints, rheumatism, bronchitis, menorrhiza, for treating rinderpest etc. [1, 4–12]. Roots of this plant yield essential oils which bring high prices due to multiple utility in modern medicine including aromatherapy [13]. The species is reported to have a great pharmaceutical importance and shown to have therapeutical and anti-inflammatory effects due to their active components like butylidene phthalide and ligustilide [14–18]. Previous work has done on essential oil of *A. glauca* from Himalayan regions (Kashmir, Himachal Pradesh and Kumaun Himalaya), which has shown great qualitative and quantitative variations in their oil compositions [19–23]. It is well established that primary and secondary metabolites such as alkaloids, terpenoids, essential oil content and composition are generally influenced by various environmental factors like habitats, altitudes, season, drying method and harvesting times [24–26].

In view of the importance of the species, a detailed and systematic study has been carried out on essential oil composition of the species, naturally growing in two locations of Garhwal Himalaya (Uttarakhand).

Fresh root of *A. glauca* was collected during the month of October, 2012 from two naturally growing areas of

Uttarakhand Himalaya (India); Kedarnath (Rudraprayag); altitude 3,400 m and Ghesh, Ghodapani (Chamoli); 3,100 m. The specimens were identified by Prof. R.D. Gaur, Department of Botany, H.N.B. Garhwal University, Srinagar Garhwal and deposited at herbarium of HAPPRC (Acc. No.: HAPPRC- AG/G SBB-1, 2).

The shade dried roots (100 g), collected from two high alpine locations were subjected to hydro-distillation for 5 h using Clevenger apparatus. The isolated essential oils were dried over anhydrous sodium sulphate and stored carefully in dark vial at low temperature before analysis.

GC analyses of the oil samples was carried out using Agilent (HP7890 GC) gas chromatograph equipped with a Flame Ionization detector (FID) and a HP-5 fused silica column (30 m × 0.32 mm, 0.25 μm film thickness). The sample was injected directly into the column. Nitrogen was used as a carrier gas during analysis. The injector and detector temperature were maintained at 210 and 230 °C, respectively. The column oven temperature was programmed from 60° to 220° with an increase in rate of 3°/min. The injection volume was 0.2 μl.

GC-MS analyses of the oils were performed on Agilent mass spectrometer (Model 5975C) coupled to an Agilent gas chromatograph with a 60 m × 0.32 mm, 0.25 μm film thickness column (DB5). The sample was injected directly in split less mode. Helium was used as the carrier gas (flow rate 1 ml/min). The oven temperature was programmed from 60° to 220° at 3 °C/min. Other conditions were the same as described under GC. The mass spectrum was taken with a mass range of 40–600 Daltons.

The identification of constituents was performed on the basis of retention index (RI), determined with reference to the homologous series of n-alkanes, $C_8$–$C_{24}$ with co-injection of standards (Sigma Aldrich USA) under same analytical conditions and by matching their recorded mass spectra with the MS library (NIST/Pfleger/Wiley) and available literature [27].

*Angelica glauca* roots yielded 0.3 and 1.8 % (v/w) volatile oils in Ghesh and Kedarnath, respectively. The content in Kedarnath sample was found to be maximum as compared to previous reports from Uttarakhand [21] and other Himalayan locations [19–23]. The percentage composition of the oils is presented in Table 1. GC and GC–MS analyses revealed an identification of 26 components, accounting 98.2 and 97.7 % in the oils from Kedarnath and Ghesh populations, respectively. The major portions in both the oils were constituted by lactone containing compounds (phthalides and ligustilides) which comprised 69.3 and 90.6 % in Ghesh and Kedarnath samples, respectively. The major constituents in the oils of *A. glauca* were characterised as (Z)-ligustilide (53.0 and 40.6 % in Kedarnath and Ghesh, respectively), (Z)-butylidene phthalide (32.8 % in Kedarnath and 20.7 % in Ghesh), (E)-butylidene phthalide (2.5 % in Kedarnath and 5.9 % in Ghesh) and (E)-ligustilide (2.3 % in Kedarnath and 2.1 % in Ghesh) and showed high degree of variation. Present study closely resembled with a report from Kumaun region of the Uttarakhand in terms of major components [21] and identified (Z)-ligustilide and (Z)-butylidene phthalide as the main compounds, constituted 54.1 and 29.8 %, respectively. Monoterpene hydrocarbons were also found in appreciable amounts in Ghesh population (19.5 %), having major compounds such as myrcene (8.3 %), α-phellandrene (6.9 %), sabinene (2.3 %) and p-cymene (0.7 %), whereas in the essential oil from Kedarnath population,

**Table 1** Constituents of *Angelica glauca* roots essential oil from two locations of Uttarakhand Himalaya (India)

| Components | RI | Composition (%) | |
|---|---|---|---|
| | | Kedarnath | Ghesh |
| α-Pinene | 935 | 0.3 | 0.2 |
| Camphene | 946 | 0.6 | – |
| Sabinene | 970 | tr | 2.3 |
| Myrcene | 986 | 0.3 | 8.3 |
| α-Phellandrene | 995 | 0.5 | 6.9 |
| p-Cymene | 1,020 | 0.3 | 0.7 |
| β-Phellandrene | 1,023 | 1.3 | 0.2 |
| Limonene | 1,024 | tr | 0.1 |
| Cis-ocimene | 1,032 | 0.2 | – |
| Trans-ocimene | 1,044 | – | 0.4 |
| γ-Terpenene | 1,049 | 0.3 | 0.4 |
| Linalool | 1,095 | tr | – |
| Terpinen-4-ol | 1,174 | 0.2 | 3.1 |
| Carvone | 1,239 | – | 0.2 |
| β-Caryophyllene | 1,423 | 0.5 | 3.4 |
| Alloraromadendrene | 1,458 | 0.6 | 0.5 |
| Germacrene-D | 1,484 | 0.1 | 0.7 |
| δ-Cadinene | 1,522 | – | 0.2 |
| Spathulenol | 1,577 | 0.2 | – |
| Epi-α-cadinol | 1,638 | tr | 0.3 |
| β-Eudesmol | 1,649 | tr | 0.5 |
| α-Cadinol | 1,652 | 2.2 | tr |
| (Z)-Butylidene phthalide | 1,671 | 32.8 | 20.7 |
| (E)-Butylidene phthalide | 1,717 | 2.5 | 5.9 |
| (Z)-Ligustilide | 1,734 | 53.0 | 40.6 |
| (E)-Ligustilide | 1,796 | 2.3 | 2.1 |
| Monoterpene hydrocarbons | | 3.8 | 19.5 |
| Oxygenated monoterpenes | | 0.2 | 3.3 |
| Sesquiterpene hydrocarbons | | 1.2 | 4.8 |
| Oxygenated sesquiterpenes | | 2.4 | 0.8 |
| Lactones | | 90.6 | 69.3 |
| Total identified (%) | | 98.2 | 97.7 |

*RI* retention index relative to n-alkanes ($C_8$–$C_{24}$) calculated on a non-polar HP-5 capillary column; *tr* trace (<0.05)

monoterpene hydrocarbons constituted only 3.8 % of the oil having comparatively lesser portions of myrcene, α-phellandrene, sabinene, p-cymene and α-pinene. β-Phellandrene which afforded 1.3 % in Kedarnath population, constituted only 0.2 % in Ghesh population. From Table 1, it is evident that the volatile oils of both the populations are different mostly quantitatively than qualitatively.

Chemical profiling studies on this species showed its great pharmaceutical importance as well as flavour and fragrance value. Previous studies revealed that butylidene phthalide, an important component of Chinese drug plants *Ligusticum wallichiana* and *Angelica sinensis* has been found to possess antispasmodic activity and improve blood flow [16, 18]. Ligustilide alleviates brain damage and improves cognitive function [15]. (Z)-ligustilide and phthalide lactones showed therapeutical effects in several diseases and possessed anti-proliferative [17] and anti-inflammatory effects [14].

Further researches still need to be done for pharmacological properties of Uttarakhand specific *Angelica* root oil. There is a need to identify the active compounds in the extracted oil, responsible for various pharmacological activities. Due to the higher relative area quantum of monomeric lactones (phthalides and ligustilides) in *A. glauca* populations growing in Uttarakhand, there is a need to develop propagation protocol for mass multiplication and in situ and ex situ conservation.

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**Conflict of Interest** The authors declare that they have no conflict of interest.

## References

1. Butola JS, Badola HK (2004) Effect of pre-sowing treatment on seed germination and seedling vigour in *Angelica glauca*, a threatened medicinal herb. Curr Sci 87:796–799
2. Vashistha RK, Butola JS, Nautiyal BP, Nautiyal MC (2010) Phenological attributes of *Angelica glauca* and *A. archangelica* expressed at two different climatic zones in Western Himalaya. Open Access J Med Aromat Plants 1:7–12
3. Anonymous (2003) CAMP report: conservation assessment and management prioritization for the medicinal plants of Jammu & Kashmir, Himachal Pradesh & Uttaranchal. FRLHT, Bangalore
4. Agrawal VS (1986) Economic plants of India. Kailash Prakasn, Calcutta, p 22
5. Anonymous (1985). The wealth of India—raw materials. Publication and Information Directorate (CSIR), New Delhi vol. 1, pp 275–276
6. Chopra RN, Nayar SL, Chopra IC (1956) Glossary of Indian medicinal plants. New Delhi, CSIR, p 330
7. Gaur RD (1999) Flora of the District Garhwal, North-West Himalaya (with ethno botanical uses). Transmedia, Srinagar, p 393
8. Jain SK, Tarafdar SS (1970) Medicinal plant-lore of the sandals. Econ Bot 24:244–245
9. Kirtikar KR, Basu BD (1988) Indian medicinal plants, Allahabad. Indian Med Plants 2:1214–1215
10. Polunin O, Stainton A (1984) Flower of the Himalaya. Oxford University Press, Delhi, p 157
11. Sharma RN, Deshpande SG, Joseph M (1990) Evaluation of natural essential oil of *Angelica glauca* Edgew. of different geographical regions. Ind Perf 34:196–198
12. Uniyal MR, Chauhan NS (1971) Medicinal plants of Vhat valley in Kangra forest. J Res India Med 6:287–299
13. Butola JS, Vashistha RK (2013) An overview on conservation and utilization of *Angelica glauca* (Edgew) in three Himalayan states of India. Med Plants 5:171–177
14. Chung JW, Choi RJ, Seo EK, Nam JW, Dong MS, Shin EM, Guo LY, Kim YS (2012) Anti-inflammatory effects of (Z)-ligustilide through suppression of mitogen-activated protein kinases and nuclear factor-kB activation pathways. Arch Pharm Res 35:723–732
15. Feng Z, Lu Y, Wu X, Zhao P, Li J, Peng B, Qian Z, Zhu L (2012) Ligustilide alleviates brain damage and improves cognitive function in rats of chronic cerebral hypoperfusion. J Ethnopharmacol 144:313–321
16. Hsieh MT, Wu CR, Lin LW, Hsieh CE, Tsai CH (2001) Reversal caused by n-butylidene phthalide from the deficits of inhibitory avoidance in rats. Planta Med 67:38–42
17. Kan WL, Cho CH, Rudd JA, Lin G (2008) Study of the anti-proliferative effects and synergy of phthalides from *Angelica sinensis* on colon cancer cells. J Ethnopharmacol 120:36–43
18. Ko WC, Chang LD, Wang GV, Lin LC (1994) Pharmacological effects of butylidene phthalidene. Phytother Res 8:321–326
19. Agnihotri VK, Thappa RK, Meena B, Kapahi BK, Saxena RK, Qazi GN, Agarwal SG (2004) Essential oil composition of aerial parts of *Angelica glauca* growing wild in North-West Himalaya, India. Phytochemistry 65:2411–2413
20. Irshad M, Rehman H, Shahid M, Aziz S, Ghous T (2011) Antioxidant, antimicrobial and phytotoxic activities of essential oil of *Angelica glauca*. Asian J Chem 23:1947–1951
21. Joshi S, Prakash O, Agarwal G, Pant AK (2005) Variations in composition of essential oil of *Angelica glauca* Edgew. root from different regions. Indian Perf 49:339–343
22. Kaul PN, Mallavarapu GR, Chamoli RP (1996) The essential oil composition of *Angelica glauca* roots. Planta Med 62:80–81
23. Thappa RK, Kaul P, Chisti AM, Kapahi BK, Suri OP, Agrawal SG (2005) Variability in the essential oil of *Angelica glauca* Edgew. of different geographical regions. J Essent Oil Res 17:361–363
24. Andola HC, Rawal RS, Bhatt ID (2011) Comparative studies on the nutritive and anti-nutritive properties of fruits in selected *Berberis* species of West Himalaya. India Food Res Int 44:2352–2356
25. Gwari G, Bhandari U, Andola HC, Lohani H, Chauhan N (2013) Volatile constituents of *Saussurea costus* roots cultivated in Uttarakhand Himalayas, India. Phcog Res 5:179–182
26. Lohani H, Haider SZ, Chauhan NK, Sah S, Andola HC (2013) Aroma profile of two *Juniperus* species from alpine region in Uttarakhand. J Nat Prod 6:38–43
27. Adams RP (2009) Identification of essential oil components by gas chromatography/mass spectrometry, 4th edn. Allured Business Media, Illinois