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Chemical composition and allelopathic, antibacterial, antifungal and *in vitro* acetylcholinesterase inhibitory activities of yarrow (*Achillea millefolium* L.) native to India

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ABSTRACT

The present study was conducted to evaluate the variability in the essential oil yield and chemical composition of *Achillea millefolium* L. (whole aerial-parts, leaves and inflorescence) grown in the subtropical region of north India during annual growth. The allelopathic, antibacterial, antifungal, and antiacetylcholinesterase activities of the oil were also examined. The essential oil yield varied due to harvesting stages and plant-parts processed (0.10–0.70%). The oils obtained from different stages and plant-parts were analysed using GC-FID and GC–MS. Altogether thirty-nine constituents, forming 93.0–97.8% of the total oil composition were identified. Major constituents of the oils were germacrene D (1.1–46.6%), sabinene (4.0–38.9%), borneol (4.7–24.9%), camphor (0.6–17.6%), α-pinene (0.8–11.7%), artemisia ketone (3.1–12.9%), chamazulene (0.8–9.6%), γ-cadinene (0.3–8.2%), and camphene (0.2–5.7%). Cluster analysis grouped overall compositions into six main groups based on their dominant components. It was observed that season of harvesting and plant-parts affect the essential oil yield and chemical composition of *A. millefolium*. The chemical composition of the examined essential oil was different from those described from other geographical origins. Moreover, this oil displayed a significant allelopathic activity.

1. Introduction

Achillea millefolium L. (Asteraceae), commonly known as 'yarrow' or 'milfoil' is a perennial herb, which grows wild all around Europe, Asia, North Africa and North America (Vitalini et al., 2011). In India, it is distributed from Kashmir to Kumaon between altitudes of 1050–3600 m (Agnihotri et al., 2005). The plant is used in fever and gastric problems by the inhabitants of the Himalayan region (Sharma et al., 2004). The plant is renowned due to its various medicinal properties, namely anti-inflammatory, antirheumatic, antispasmodic, antiseptic, carminative, astringent, digestive, diaphoretic, expectorant, tonic, haemostatic, stomachic *etc*. (Judzentiene, 2016). *A. millefolium* has been extensively used in folk and modern medicines, veterinary, food and cosmetic industry (Judzentiene, 2016). Its medicinal properties have been recognized globally. It has been included in the national Pharmacopoeias of various European countries, namely Germany, Czech Republic, France and Switzerland (Cavalcanti et al., 2006). Moreover, the essential oil of the plant has been recognized because of its anti-inflammatory and disinfectant properties (Nadim et al., 2011).

Phytochemical studies carried out so far indicate the presence of a remarkable chemical polymorphism within *A. millefolium* populations. Main bioactive compounds of the plant are flavonoids (apeginenin, rutin, lutelin, campherol) and essential oil (Bimbiraite et al., 2008). The chemistry of the essential oil of *A. millefolium* is very complex due to the existence of a number of chemotypes (Gudaityte and Venskutonis, 2007; Raal et al., 2012). Major components in the oils isolated from different chemotypes are chamazulene, sabinene, β-pinene, 1,8-cineole, linalool, α-thujone, β-thujone, ocimene, camphor, ascaridole, caryophyllene oxide, β-eudesmol and α-bisabolol (Mockute and Judzentiene, 2003). The essential oil yield and chemical composition of the plant vary significantly due to various extrinsic and intrinsic factors

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(Bimbiraite et al., 2008; Figueiredo et al., 2008). Diploid and tetraploid populations of *A. millefolium* possessed proazulene sesquiterpenes, which transformed to chamazulene (up to 25%) during the process of hydrodistillation. Further, the main constituents of the hexaploid populations are camphor, sabinene, 1,8-cineole *etc*., however, a major component of the octoploid populations is linalool (Raal et al., 2012).

The information on the essential oil chemistry of *A. millefolium* is meager from India. Populations growing naturally in Jammu & Kashmir and Himachal Pradesh have been subjected to preliminary screening, but no systematic work has been done so far to ascertain its value for sustainable industrial utilization (Agnihotri et al., 2005; Nadim et al., 2011). Moreover, a review of the literature revealed that there are no previous studies regarding the exploration of essential oil variability due to plant-parts and season of harvesting from India. Therefore, the aim of this study was to explore the variability in the essential oil profile occurring due to harvesting seasons/growth stage and plant-parts processed, and to assess the allelopathic, antibacterial, antifungal, and acetylcholinesterase inhibitory activities of *A. millefolium* native to the Uttarakhand region of India.

2. Materials and methods

2.1. Plant material

Achillea millefolium (white-flowered, blue oil type population) was planted in the experimental field of CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre Pantnagar, Uttarakhand, India in the month of July 2015. The samples of fresh biomasses, *viz*. whole aerial-parts (*ap*: according to the plant stage it consisted of leaves/inflorescence/stem or leaves/stem), leaves (*l*) and inflorescence (*i*) was collected at different stages of plant growth during 2015–2016 (Table 1). Authentication of the plant material was done by one of the authors (AC) and the voucher specimen has been retained at the departmental herbarium. The experimental site is located between at 29° N and 79.38° E, and at an altitude of 243 m above mean sea level, experiencing the subtropical, humid climate. The maximum temperature ranges between 35 and 45 °C, and minimum between 2 and 5 °C. The soil of the experimental site is sandy-loam, with neutral pH.

2.2. Isolation of the essential oil

The samples of the freshly harvested biomasses, *viz*. whole aerial-parts, leaves and inflorescence of *A. millefolium* was separately subjected to hydrodistillation (3 h) in a Clevenger apparatus for isolation of their essential oils. The obtained essential oils were dried over anhydrous sodium sulphate and yields (%) determined on fresh weight basis. All oil samples were kept in the refrigerator until their further analysis.

2.3. Quantification and characterization of essential oil (GC-FID and GC–MS)

Quantification and characterization of the essential oil constituents were carried out by GC-FID and GC–MS techniques as per the procedures described earlier (Verma et al., 2014). Briefly, GC-FID was performed on Nucon gas chromatograph (model 5765) equipped with DB-5 fused-silica capillary column (30 m × 0.25 mm id; 0.25 μm film thickness). GC–MS (EI 70 eV; mass scan range 40–400 amu) was recorded on Clarus 680 GC interfaced with a Clarus SQ 8C mass spectrometer of PerkinElmer equipped with Elite-5 MS fused-silica capillary column (30 m × 0.25 mm id; 0.25 μm film coating). Identification of the constituents was carried out by comparing the observed retention index (RI) and mass spectra with MS Library (NIST and WILEY) and literature (Adams, 2007).

2.4. Allelopathic bioassay

Germination, and root and shoot length elongation of test crop seeds (*Lactuca sativa*) were evaluated in petri-dishes (9.0 cm diameter × 1.5 cm height) under aseptic conditions with three replications, each containing 30 seeds. Thirty surface sterilized seeds of lettuce were placed separately in a sterilized bioassay system. The filter paper in each petri-dish was moistened with distilled water and essential oil (whole aerial-parts; S-6) containing paper strip adhered inside the lid of the petri-dish. The data pertaining to seed germination, root length, and shoot length were recorded after seven days of sowing. All the experimental observations were performed in triplicate.

2.5. Antibacterial assays (disc diffusion assay and microdilution broth assay)

The antibacterial activity of the essential oil (whole aerial-parts; S-6) was determined against *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 435), *Streptococcus mutans* (MTCC 890), *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aerugenosa* (MTCC 741), *Escherichia coli* (MTCC 723) and *Salmonella typhimurium*

Table 1
Effect of collection time and plant-parts on the essential oil yield of *Achillea millefolium* grown in the subtropical region of north India.

Month of collection	Plant stage	Stage code	Essential oil yield (%)[a]		
			Whole aerial-parts (*ap*)	Leaf (*l*)	Inflorescence (*i*)
October	Vegetative stage	S-1	0.16 ± 0.01	0.16 ± 0.01	–
November	Vegetative stage	S-2	0.16 ± 0.01	0.16 ± 0.01	–
December	Flowering initiation	S-3	0.14 ± 0.01	0.10 ± 0.02	0.47 ± 0.02
January	Flowering stage	S-4	0.14 ± 0.01	0.12 ± 0.02	0.49 ± 0.02
February	Flowering stage	S-5	0.14 ± 0.01	0.12 ± 0.01	0.50 ± 0.02
March	Flowering stage	S-6	0.16 ± 0.02	0.19 ± 0.01	0.59 ± 0.01
April	Flowering stage	S-7	0.16 ± 0.01	0.16 ± 0.01	0.50 ± 0.02
May	Late-flowering stage: half mature	S-8	0.15 ± 0.01	0.24 ± 0.02	0.70 ± 0.04
June	Late-flowering stage: full mature	S-9	0.15 ± 0.01	0.16 ± 0.02	0.58 ± 0.02
July	Late-flowering stage: seed shattering	S-10	0.13 ± 0.01	0.12 ± 0.01	0.26 ± 0.02

[a] Determined on fresh weight basis (mean ± standard deviation, n = 3).