Industrial Crops and Products 42 (2013) 195–201

Contents lists available at SciVerse ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Phytochemical analysis of the leaf volatile oil of walnut tree (*Juglans regia* L.) from western Himalaya

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ARTICLE INFO

Article history:
Received 29 January 2012
Received in revised form 18 April 2012
Accepted 26 May 2012

Keywords:
Juglans regia
Walnut tree
Juglandaceae
Leaf essential oil
Chemical diversity
GC/MS

ABSTRACT

The walnut tree (*Juglans regia* L.), commonly known as 'Akhrot' in India, is a valuable tree has a long history of medicinal use to treat a wide range of health complaints. To explore the diversity in essential oil yield and composition of *J. regia*, leaves were collected during spring season from 28 populations growing in western Himalaya. Comparative results showed considerable variations in the essential oil yield and composition of *J. regia* leaves. The essential oil yield varied from 0.02% to 0.12% in fresh leaves of the different populations of *J. regia*. Analysis of the essential oils by GC/FID and GC/MS and the subsequent classification by statistical analysis resulted in three clusters with significant variations in their terpenoid composition. Altogether, 70 constituents, representing 83.2–98.0% of the total oil composition, were identified and quantified. Major components of the essential oils were (*E*)-caryophyllene (1.4–47.9%), *β*-pinene (4.5–39.5%), germacrene D (5.0–23.3%), *α*-pinene (1.5–18.1%), *α*-humulene (1.1–11.8%), *α*-zingiberene (0.1–11.3%), *α*-copaene (0.0–10.1%), limonene (0.8–8.6%), caryophyllene oxide (0.1–8.6%), *ar*-curcumene (0.0–7.2%), *δ*-cadinene (0.3–6.7%), (*E*)-*β*-farnesene (0.0–5.9%), 1,8-cineole (<0.0–5.4%), *γ*-curcumene (0.0–4.2%), and methyl salicylate (0.1–4.0%). This is the first report on leaf volatile oil composition of *J. regia* populations from western Himalaya. Out of the 70 identified constituents, over 25 were described for the first time for *J. regia*.

1. Introduction

Walnut tree (*Juglans regia* L.), belongs to family Juglandaceae, is native to south-eastern Europe, Asia Minor, India, and China. In India it is commonly known as 'Akhrot' and grows in Himalaya up to an altitude of 900–3300 m. Some species of the walnut tree is now cultivated in Europe, North America, North Africa, and East Asia (Tsamouris et al., 2002). This valuable tree has a long history of medicinal use to treat a wide range of health complaints. Almost all parts of the plant are medicinally important. Dry seeds (nuts) are very popular and largely consumed as royal food in whole country. In addition to it, green walnuts, shells, bark, green husks (epicarps), and leaves have been used in the cosmetic and pharmaceutical industries (Oliveira et al., 2008). The stem bark is reported to be alterative, anthelmintic, astringent, bactericide, depurative, digestive, diuretic, laxative, detergent, stimulant, tonic, and insecticidal (Chopra et al., 1986). The walnut oil is a component of dry skin creams, antiwrinkle, and antiaging products, because it presents moisturizing properties as well as free radical scavenging capacity (Espin et al., 2000). Leaves of the *J. regia* have been widely used in folk medicine for treatment of venous insufficiency and haemorrhoidal symptomatology, for their antidiarrheic, antihelmintic, depurative, and astringent properties, and also leaves mixed with stored-grains as fungicide and insecticide (Cosmulescu and Trandafir, 2011; Negi and Kanwal, 2009). Seed cover of *J. regia* is being used by Bhotiya ethnic groups to produce 'camel' colour (Kala, 2002). Other properties such as antibacterial, human cancer cell antiproliferative, antioxidant, keratolytic, antifungal, hypoglycaemic, hypotensive, anti-scrofulous, and sedative have also reported for this plant (Carvalho et al., 2010; Gırzu et al., 1998; Pereira et al., 2007; Valnet, 1992). The nuts are rich in unsaturated fatty acids (linoleic acid and oleic acid), protein (arginine, leucine), carbohydrates (dietary fibre), vitamins (vitamin A, C, E), pectic substances, minerals (magnesium, potassium, phosphorus, sulphur, copper, and iron), fibres, melatonin, plant sterols, phenolic acids, and flavonoids (Chopra et al., 1986; Kris-Etherton et al., 1999; Labuckas et al., 2008; Pereira et al., 2008; Prasad, 2003). Juglone (5-hydroxy-1,4-naphthoquinone), which occurs in different plant parts of *J. regia*, is a well known allelopathic agent and being used in commercial hair dye and also has therapeutic properties (Thakur, 2011).

Literature survey revealed that a lot of research work has been carried out on non-volatile phytochemicals of *J. regia*

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http://dx.doi.org/10.1016/j.indcrop.2012.05.032

Table 1
Origin and essential oil yield of Indian Walnut (*Juglans regia*) populations.

Population code	Location	Coordinates	Altitude (ft)	Essential content [%][a]
JR1	Vijaypur (Bageshwar)	N 29°50.362′; E 079°55.135′	5510 (±103)	0.04
JR2	Chhati (Bageshwar)	N 29°49.514′; E 079°48.882′	5331 (±37)	0.04
JR3	Ghinghartola (Bageshwar)	N 29°50.023′; E 079°51.111′	5480 (±34)	0.02
JR4	Kotmanya (Bageshwar)	–	–	0.02
JR5	Sirkote (Bageshwar)	N 29°57.956′; E 079°34.357′	5324 (±43)	0.02
JR6	Kandhar (Bageshwar)	N 29°56.758′; E 079°35.124′	4331 (±28)	0.02
JR7	Dewalchaura (Bageshwar)	N 29°53.532′; E 079°47.706′	3150 (±29)	0.02
JR8	Ashon (Kapkote, Bageshwar)	N 29°55.855′; E 079°53.287′	3212 (±49)	0.03
JR9	Harsila (Kapkote, Bageshwar)	N 29°54.434′; E 079°48.871′	3202 (±52)	0.02
JR10	Shama (Kapkote, Bageshwar)	N 29°58.115′; E 080°02.506′	6747 (±64)	0.07
JR11	Laubanj (Bageshwar)	N 29°52.560′; E 079°35.511′	3815 (±31)	0.05
JR12	Aeiradi (Pithoragarh)	N 29°50.823′; E 079°59.666′	6534 (±74)	0.03
JR13	Devinagar (Pithoragarh)	N 29°47.472′; E 080°02.234′	6637 (±103)	0.04
JR14	Dewalthal (Pithoragarh)	–	–	0.06
JR15	Gwaldam (Chamoli)	N 30°00.496′; E 079°33.394′	6445 (±103)	0.03
JR16	Tharali (Chamoli)	N 30°04.299′; E 079°30.005′	4107 (±34)	0.03
JR17	Manan (Almora)	N 29°43.419′; E 079°37.081′	4272 (±42)	0.04
JR18	Tarikhet (Almora)	–	–	0.08
JR19	Dinga (Almora)	–	–	0.08
JR20	Lohali (Nainital)	N 29°29.790′ E 079°30.135′	3098 (±29)	0.03
JR21	Vinayak, (Nainital)	N 29°21.992′ E 079°33.163′	4668 (±48)	0.05
JR22	Padampuri (Nainital)	N 29°22.872′ E 079°37.166′	5233 (±52)	0.05
JR23	Dhanachuli (Nainital)	N 29°23.794′ E 079°39.427′	6992 (±58)	0.05
JR24	Pahadpani (Nainital)	N 29°25.425′ E 079°42.511′	6859 (±32)	0.04
JR25	Berchula (Nainital)	N 29°25.929′ E 079°49.266′	6590 (±75)	0.03
JR26	Batiya (Nainital)	–	–	0.09
JR27	Richi (Nainital)	–	–	0.12
JR28	Devidhura (Champawat)	N 29°24.556′ E 079°51.867′	6013 (±53)	0.03

[a] Essential oil calculated on fresh weight of leaves.

(Fukuda et al., 2003; Ito et al., 2007; Thakur, 2011; Zhang et al., 2009). However, information concerning volatile oil composition of *J. regia* is meagre. Volatile chemical constituents of the mature nuts and green walnut husks of *J. regia* have been investigated (Abbasi et al., 2010; Buttery et al., 2000; Elmore et al., 2005). A single report on head space analysis of volatile compounds of *J. regia* leaves from Egypt is also available (Farag, 2008). However, to the best of our knowledge leaf volatile oil (essential oil) composition of *J. regia* has yet not been investigated from Himalayan populations. Therefore, in present investigation leaf volatile oil composition of *J. regia*, collected from 28 different locations of western Himalaya have been compared by chromatographic analysis and classified based on clustering pattern derived by statistical analysis.

2. Materials and methods

2.1. Plant materials

To avoid variability in essential oil yield and composition due to season, fresh leaves of *J. regia* were collected during spring season from 28 different locations of western Himalaya, India. The investigated populations of *J. regia* were authenticated by one of the author (AC) and voucher specimen of the plant is kept in the Herbarium of CIMAP Research Centre, Pantnagar, India. The origin (location name, coordinates, and altitude) of different populations of *J. regia* is summarized in Table 1. The leaves of the all collected samples were separated from stem and used for isolation of volatile oils.

2.2. Isolation of the volatile oils

The fresh leaves (moisture, 69.4%) of *J. regia* were subjected to hydro-distillation in a Clevenger type apparatus for 3 h for isolation of essential oil. Essential oil was measured directly in the isolation burette and content (%) was calculated as volume (ml) of essential oil per 100 g of fresh plant material. The oils were dehydrated by anhydrous Na_2SO_4 and kept in a cool and dark place prior to analysis.

2.3. Gas chromatography (GC)

GC analysis of the essential oils derived from *J. regia* populations was carried out on *Nucon* gas chromatograph model *5765* (Aimil-Nucon, New Delhi) equipped with flame ionization detector (FID) and DB-5 (30 m × 0.32 mm, 0.25 μm film coating) fused silica capillary columns. Hydrogen was used as carrier gas at 1.0 ml/min. Oven temperature programming was done from 60 to 230 °C at 3 °C/min with final hold time of 10 min. The injector and detector temperatures were 220 °C and 230 °C, respectively. The injection volume was 0.02 μl neat and split ratio was 1:40.

2.4. Gas chromatography–mass spectrometry (GC–MS)

GC–MS analysis of the essential oil was carried out on a PerkinElmer AutoSystem XL GC interfaced with a Turbomass Quadrupole mass spectrometer fitted with an Equity-5 fused silica capillary column (60 m × 0.32 mm i.d., film thickness 0.25 μm; Supelco Bellefonte, PA, USA). The column temperature was programmed 70 °C, initial hold time of 2 min, to 250 °C at 3 °C/min with final hold time of 2 min. The injector temperature was 220 °C, transfer line and source temperatures were 250 °C. Injection size 0.03 μL neat; split ratio 1:30; carrier gas He at 10 psi constant pressure; ionization energy 70 eV; mass scan range 40–450 amu.

2.5. Identification of compounds

Identification of the essential oil constituents was carried out on the basis of retention index (determined with reference to homologous series of *n*-alkanes, C_8–C_{30}), MS Library search (NIST/EPA/NIH version 2.1 and WILEY registry of MS data 7th edition), by comparing with the MS literature data (Adams, 2007). The retention times of marker constituents of known essential oils were also used to confirm the identities of constituents (α-pinene,