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In vitro screening of essential oil from young and mature leaves of *Artemisia scoparia* compared to its major constituents for free radical scavenging activity

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ABSTRACT

The present study investigated the chemical characterization, and antioxidant activity of essential oil hydrodistilled from young and mature leaves of *Artemisia scoparia*. GC–MS analyses revealed a monoterpenoid nature (64–67%) with 44 and 31 constituents in young and mature leaves oil, respectively. The oil from young leaf contained greater amount of oxygenated compounds. β-Myrcene (24.13%) and *p*-cymene (27.06%) were the major constituents in young and mature leaves oil, respectively. *A. scoparia* leaf oils (25–200 μg/ml) exhibited a strong 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity and antioxidant activity against hydroxyl radical and hydrogen peroxide. However, the activities of major constituent monoterpenes, β-myrcene and *p*-cymene, were less. In general, the DPPH radical scavenging and antioxidant activity was in the order: mature leaf oil > young leaf oil > β-myrcene > *p*-cymene.

1. Introduction

Reactive oxygen species (ROS: superoxide anion radicals, $O_2^{\bullet -}$; hydroxyl radicals, $OH^{\bullet}$; hydrogen peroxide, H_2O_2; and singlet oxygen, $^1O_2$) are the chemically reactive ions, generated as byproducts of primary metabolic activities. Excess of ROS/free radicals damage enzymatic machinery, oxidize carbohydrates, proteins, lipids and DNA, and thus induce disease and cellular injury (Halliwell and Gutteridge, 1999). The deleterious effects of ROS-mediated cellular injury have aroused the attention for the search of antioxidants that can be supplemented into the dietary foodstuffs. To scavenge free radicals, reduce lipid peroxidation, and prevent microbial degradation of food, synthetic antioxidants (BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene) have been widely used as food additives (Pokorný, 2007). However, due to increasing awareness among people for the use of safer compounds there has been a shift towards the use of natural compounds as antioxidants (Singh et al., 2008b). In this direction, essential oils and their pure components due to their non-toxic nature and a wide spectrum of biological activities (Batish et al., 2008), and potential to control the free radicals are now being explored as flavoring agents in foods (Bakkali et al., 2008). In fact, essential oil from various aromatic plants has been a subject of intense research due to their multifunctional uses other than classical roles as raw material in pharmaceuticals, food industries and perfumeries. These include: antimicrobial, antifungal, insecticidal, insect-repellant, bioherbicidal, and free radical scavenging activity (Batish et al., 2004, 2008; Bakkali et al., 2008; Isman, 2006; Ramezani et al., 2002; Singh et al., 2005; Singh et al., 2008a, 2008b; Wei and Shibamoto, 2007). In fact, these volatiles/essential oils provide an important defense strategy to plants and also depict an evolutionary relationship with their functional roles in plants *per se* (Batish et al., 2008).

Artemisia scoparia (redstem wormwood; Asteraceae) is a faintly scented annual herb widespread and common throughout the world, particularly Southwest Asia and Central Europe (Anonymous, 1993). In India, it is abundant in western Himalayas (up to 2100 m), Punjab, and upper Gangetic plains. The success of *A. scoparia* may be attributed to presence of phytotoxins – the volatile essential oil besides the other nonvolatile secondary products (Singh et al., 2008a; Singh et al., 2009a). It has been established that aerial parts of *A. scoparia* yield volatile essential oil that has medicinal value (Anonymous, 1993) and insecticidal activity (Negahban et al., 2006). It possesses antibacterial, anticholesterolemic, antipyretic, antiseptic, cholagogue, diuretic, purgative, and vasodilator activity, and is also used for treatment of gall bladder inflammation, hepatitis, and jaundice (Yeung, 1985). Earlier, researchers have documented that volatile oil from *A. scoparia* is rich in oxygenated monoterpenoids and the chemical composition varies significantly with geographical region (Cha et al., 2005; Safaei-Ghomi et al., 2005; Mirjalili et al., 2007), yet no study has been undertaken to evaluate the variability in chemical constituents and the antioxidant activity of the essential oil with leaf age/growth stage. Therefore, a study was planned to extract and characterize essential oil

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from young and mature leaves of *A. scoparia*, and determine the free radical scavenging and antioxidant activities of oil and its major constituent.

2. Materials and methods

2.1. Extraction of essential oil

Essential oil was extracted from freshly plucked leaves of *A. scoparia* Waldst. & Kit. (redstem wormwood) by hydrodistillation using Clevenger's apparatus (Singh et al., 2009b). The young and mature leaves were separated from the plants growing in wastelands around Chandigarh (India). The two types of leaves differed in pigment (chlorophyll) and protein content. Total chlorophyll content was found to be higher in mature leaves (3.21 ± 0.58 μg/g) compared to young leaves (2.54 ± 0.24 μg/g). Likewise, the content of total proteins was higher in mature leaves (397.2 ± 6.2 mg/g) over that in young leaves (334.2 ± 14.5 mg/g). Young or mature leaves (250 g) were mixed with 500 ml distilled water in a round bottom flask fitted with condenser, and boiled for 3 h. The clear yellow colored oil was collected from the nozzle of the condenser. It was dried over sodium sulphate and stored at 4 °C for further use.

2.2. GC/GC–MS analyses of the oils

Essential oil was analyzed by Gas Chromatography (GC) and Gas Chromatography–Mass Spectroscopy (GC–MS) for identification as per Singh et al. (2008b).

GC was performed on Shimadzu GC-17A Gas Chromatograph equipped with a flame ionization detector (FID) and a capillary column type DB-5 (60 m × 0.25 mm, i.d., membrane thickness 0.25 μm). He (helium) was used as carrier gas at a flow rate 1 ml min^{-1} and flux ratio was set at 20:1. The injector and detector temperature were kept at 250 °C and 280 °C, respectively. Initially, the temperature was set at 50 °C for 2 min, then programmed at 4 °C min^{-1} to 260 °C, and held at this temperature for 3 min. Relative amount of different constituents were determined by computerized peak area normalization without any correction factor of three injections, and comparison with GC–MS data.

GC–MS analysis was performed on a Shimadzu QP 2010 Mass Spectrophotometer fitted with fused silica (SGE BP 20) capillary column (30 m × 0.25 mm, i.d., 25 μm film thickness). Helium (He) was used as carrier gas with a split ratio of 1:50 and a linear velocity of 38.5 cm s^{-1}. The injector and detector temperature were 220 °C and 250 °C, respectively. Initially, the temperature was set at 70 °C (held for 4 min), programmed to 220 °C at 4 °C min^{-1}, and held for 5 min. The mass spectral range was recorded from *m/z* 40 to 600 amu.

2.3. Identification of constituents in the oils

The identification of the various components was done on the basis of (1) co-elution and comparison of their retention times with those of pure authentic samples purchased from Sigma–Aldrich (St. Louis, USA), Fluka (Buchs, Switzerland), Acros (Geel, Belgium) and AlfaAeser (Massachusetts, USA), (2) comparison of their retention indices (RI) with reference to homologus series of *n*-alkanes (C_7–C_{30}; Supelco, Bellefonte, PA, USA), (3) computer matching of mass spectra using library search system HP-5872, and consulting data bases of Wiley 275 and NBS 75 K Libraries (McLafferty, 1989), NIST 98 (Stein, 1998), and compilation by Adams (1995).

2.4. Procurement of materials

The principle components β-myrcene and *p*-cymene of *Artemisia* oil from young and mature leaves, respectively, were purchased from Sigma–Aldrich (St. Louis, USA), and AlfaAeser (Massachusetts, USA). All other chemicals used in analysis were of analytical grade and purchased locally from best available sources (Sisco Research Laboratory, Mumbai, India; Loba-Chemie, Mumbai; Sigma–Aldrich, St. Louis, USA).

2.5. Free radical scavenging capacity (RSC)

RSC of *Artemisia* leaf essential oils and two major constituent monoterpenes (β-myrcene and *p*-cymene) was evaluated by measuring scavenging activity against DPPH (2,2-diphenyl-1-picrylhydrazil) radical as per Bozin et al. (2006). The oils/monoterpene (25–200 μg/ml) solutions were mixed into 1 ml of DPPH (90 μM, in methanol) and the final volume was made to 4 ml with methanol. After 1 h of incubation in dark at room temperature, the absorbance of the solutions including blank (without sample) and a positive control (BHT, *tert*-butylated hydroxytoluene) was read at 515 nm on UV–VIS spectrophotometer (Shimadzu UV-190). For each sample assay, minimum three replicates were maintained, and data presented as mean of three. A decrease in absorbance of DPPH solution indicates increased RSC and was calculated in percent using the following formula:

$$\%\ \text{DPPH radical scavenging} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

2.6. Hydroxyl radical ($OH^{\bullet}$) scavenging

It was examined by measuring the competition between 2-deoxyribose and the essential oil for $OH^{\bullet}$ generated in a Fenton reaction. $OH^{\bullet}$ degrades 2-deoxyribose to form thiobarbituric acid reactive substance (TBARS) that could be measured at 532 nm (Bozin et al., 2006). The reaction mixture contained 3 mM 2-deoxyribose, 0.1 mM $FeCl_3$, 1 mM H_2O_2, 0.1 mM EDTA, 0.1 mM ascorbic acid, and 0.02 M phosphate buffer (pH 7.4). The oils or monoterpene (25–200 μg/ml) solutions were added to 3 ml of reaction mixture. After 1 h incubation at 37 °C, 1 ml of TBA (1%) and 1 ml of trichloroacetic acid (2.8%) were added, and the mixture was heated at 100 °C for 20 min. After cooling the mixture, absorbance was read at 532 nm against a blank containing buffer and 2-deoxyribose. The percent inhibition (*I*) of deoxyribose degradation was measured using the formula:

$$\%\ I = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

2.7. Hydrogen peroxide (H_2O_2) scavenging

It was determined using 40 mM H_2O_2 prepared in phosphate buffer (pH 7.4) following Ruch et al. (1989). The concentration of H_2O_2 was determined spectrophotometrically (230 nm) 10 min after adding sample solutions (25–200 μg/ml) to 0.6 ml of H_2O_2 (40 mM). The solution without $H_2O_2$was used as blank. The percent H_2O_2 scavenging was determined using the formula:

$$\%\ \text{Scavenging of } H_2O_2 = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

3. Results and discussion

3.1. GC–MS analysis

The young leaves of *A. scoparia* upon steam distillation yielded ~0.20% (v/w) of essential oil, whereas the oil yield from mature leaves was ~0.24%. The oils from freshly collected young and mature leaves contained 44 and 31 compounds constituting ~98.38% and 99.99% of the oils, respectively (Table 1). The essential oil from both young and mature leaves was mainly monoterpenoid in nature with 23 and 18 monoterpenes accounting for 64% and 67% of the oils, respectively (Table 2). Nearly 15% of the monoterpenes in young leaf oil were oxygenated in nature. In addition, the oil from young leaf contained ~13% of sesquiterpenes, of which 12% were oxygenated in nature (Table 2). In contrast, the oil from mature leaf contained only 2.3% sesquiterpenes (Table 2).

In the young leaf oil, β-myrcene (24.13%) was the major constituent monoterpene, whilst *p*-cymene (27.06%) was the major component in mature leaf oil (Table 1). The other major monoterpene constituents in young leaf oil included monoterpenes such as *p*-cymene (16.47%), (+)-limonene (8.03%), and oxygenated compounds such as caryophyllene oxide (a sesquiterpene; 7.86%) and capillin (a polyacetylene ketone; 7.13%) (Table 1). In contrast, the mature leaf oil contained β-myrcene (20.89%) as the second major constituent. The young leaf oil contained >12% oxygenated sesquiterpenes such as caryophyllene oxide, isocaryophyllene oxide, *trans*-nerolidol, (−)-spathulenol, humulene epoxide II, 5-*neo*-cedrol (Table 1). These constituents were either absent or present in very less amount in the oil from mature leaves. Though the exact reason for such an observation is unknown, yet it is opined that, in general, the oxygenated compounds in the young leaf get transformed into the monoterpene hydrocarbons with age. As a result, mature leaf oil contains lesser amount of oxygenated compounds and more of the hydrocarbons. Further, the oil from young leaves contained 7.13% of capillin, which was, however, absent in the mature leaves. The presence of capillin and greater amount of oxygenated sesquiterpenes in the oil from young leaf of *A. scoparia* from