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Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

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

Original article http://dx.doi.org/10.1016/j.apjtb.2016.07.010

Chemical diversity of essential oils from flowers, leaves, and stems of *Rhanterium epapposum* Oliv. growing in northern border region of Saudi Arabia

Marwa Awad[1,2*], Abdelrhman Abdelwahab[1,3]

[1]*Department of Biological Science, Faculty of Science, Northern Border University, Arar, Saudi Arabia*

[2]*Department of Medicinal and Aromatic Plants, Phytochemical Units, Desert Research Center, Cairo, Egypt*

[3]*Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Cairo, Egypt*

ARTICLE INFO

Article history:
Received 8 Jan 2016
Received in revised form 7 Feb, 2nd revised on 15 Mar, 3rd revised on 21 Mar 2016
Accepted 18 May 2016
Available online 30 Jul 2016

Keywords:
Rhanterium epapposum Oliv.
Asteraceae
Essential oils
Monoterpenes

ABSTRACT

Objective: To evaluate the medicinal uses of *Rhanterium epapposum* Oliv. (*R. epapposum*) growing in northern border region of Saudi Arabia, through the chemical diversity of essential oils extracted from its flowers, leaves and stems.

Methods: Aerial parts of *R. epapposum* were collected in April 2014. Air dried flowers, leaves, and stems were separately subjected to hydrodistillation in a Clevenger-type apparatus for 4 h to extract the essential oils. Gas chromatography-mass spectrometry analysis of the essential oils was carried out using an Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass spectrometric detector.

Results: A total of 51 compounds representing 76.35%–94.86% of flowers, leaves and stems oils composition were identified. The chemical profiles of the studied fractions revealed the dominance of monoterpenes, regardless of qualitative and quantitative differences observed. Limonene, linalool, 4-terpineol and α-cadinol represented the major constituents of flowers oil. Leaves oil was dominated by limonene, sabinene, α-pinene and β-myrcene whereas linalool, ionole, α-cadinol, β-eudesmol, 4-terpineol, and α-terpineol were the major constituents of stems oil.

Conclusions: Essential oils from flowers, leaves and stems of *R. epapposum* growing in northern border region of Saudi Arabia are considered as a rich source of monoterpenes which have biological activities.

1. Introduction

Plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases. The knowledge of their healing properties has been transmitted over centuries within and among human communities. Active secondary metabolites are usually responsible for the biological properties of some plant species used throughout the globe for various purposes, including treatment of infectious diseases [1]. Plants essential oils are natural compounds that have multi-purpose applications [2]. In pharmaceutical industry, the essential oils have been used due to their anticancer, antinociceptive, antiphlogistic, antiviral, antibacterial, and antioxidant properties [3]. They have other uses in food and cosmetic industry [2,4,5].

Genus *Rhanterium* (family Asteraceae, tribe Inuleae) is represented by several species namely *Rhanterium adpressum* (*R. adpressum*), *Rhanterium apressum*, *Rhanterium epapposum* (*R. epapposum*), *Rhanterium incrassatum*, *Rhanterium squarrosum*, and *Rhanterium suaveolens* [6]. *Rhanterium* species are globally distributed over Western, North Africa, Afro–Asian countries, the Arabian Peninsula, Iraq and Iran [7,8]. In Saudi Arabia, genus *Rhanterium* is represented by *R. epapposum*, which is a perennial dwarf shrub, with richly branched pale stem, up to 70 cm height, tiny narrow leaves and yellow flowers 1.5 cm wide, cupped in a soft-spiny involucre [9–11]. *R. epapposum* is distributed in Saudi Arabia mainly in northern region [9–11], Kuwait [12], north-eastern parts of the United Arab Emirates [13], Iran [7] and Sudan [8].

*Corresponding author: Marwa Awad, Department of Biological Science, Faculty of Science, Northern Border University, P.O. Box 1321, Arar 91431, Saudi Arabia.
Tel: +966541808037
E-mail: dr_marwahassan@yahoo.com

Foundation Project: Supported by Deanship of Scientific Research, Northern Border University, Saudi Arabia (Grant No. 434/39).

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

R. epapposum, locally known as "Al-Arfaj", is used in folk medicine by people in rural areas as a remedy for skin infections, gastrointestinal disturbances and as an insecticide [8,14]. To our knowledge, there is only one report on the composition of the essential oil of *R. epapposum* having been published [15]. The researchers investigated the essential oil from aerial parts of *R. epapposum* growing in Northeastern Iran by thin layer chromatography, liquid–solid chromatography, capillary gas liquid chromatography, and gas liquid chromatography-mass spectrometer. The oil contained 107 volatile components of which 92% were terpenoids. The main constituents were α-phellandrene, linalol, geraniol, bulnesol, and β-phellandrene accounting for 55.6% of the oil. Non-terpenoid aliphatic and aromatic structures have been detected.

Regarding the other species of genus *Rhanterium*, the chemical composition of the essential oil of the aerial parts of *R. adpressum* Coss. & Durieu from Algeria was investigated [7]. The oil was characterized by the presence of high amounts of spathulenol (19.6%), β-eudesmol (15.2%), bicyclo[4.4.0] dec-1-ene, 2-isopropyl-5methyl-9-methylene (12.9%), β-cadinol (11.3%), α-cadinol (6.56%), α-eudesmol (5.37%), myristicin (5.05%), 2-H-pyran-3-ol, and tetrahydro-2-(1,7-nonadiene-3,5-diynyl) (4.81%). In general, the volatile oil is a rich source of sesquiterpenic compounds. Another study has been focused on essential oil of the flowers of the same species [16]. The main constituents of the oil were monoterpene hydrocarbons: camphene (21.8%), myrcene (19.3%) and α-pinene (17.4%). Other compounds, including limonene, β-pinene and terpinol-4-ol, were present in low content (4%–6%). The composition of the fatty acids in the lipid extract obtained from the flowers was also investigated by gas chromatography and gas chromatography-mass spectrometry (GC–MS). The main fatty acids identified were palmitic (47.4%), oleic (12.9%) and stearic acids (10.6%).

Other phytochemical constituents of genus *Rhanterium* had been investigated [17–23]. In respect to biological activities of *R. epapposum*, petroleum ether and methanol extracts showed antibacterial activity [18] and methanolic extract showed 3.3% diarrhea inhibition [17].

According to our knowledge, there are no previous reports about the composition of the essential oil of *R. epapposum* in Saudi Arabia. The present study was planned to evaluate the medicinal uses of *R. epapposum* from northern border region of Saudi Arabia, through the chemical diversity of essential oils extracted from its flowers, leaves and stems.

2. Materials and methods

2.1. Plant material

Aerial parts of *R. epapposum* were collected from Dawmat-Algandal region, located at the northern border region of Saudi Arabia in April 2014. Identification of plant material was based on Chaudhary, Collenette, and Migahid [9–11]. Voucher specimen (Ref. No. 78) was deposited in the herbarium of Faculty of Science, Northern Border University.

2.2. Isolation of the essential oils

Air dried flowers (100 g), leaves (100 g) and stems (1 000 g) of *R. epapposum* were separately subjected to hydrodistillation in a Clevenger-type apparatus for 4 h according to the British Pharmacopoeia specification [24]. The essential oils' content (v/w) was estimated on a dry weight basis. The oil samples obtained were dehydrated over anhydrous sodium sulfate and stored at 4 °C in dark for analysis.

2.3. GC–MS analysis

GC–MS analyses of the essential oils were performed using an Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass spectrometric detector, with a direct capillary column Hp-5ms (30 m × 0.32 mm × 0.25 μm film thickness). Samples were injected under the following conditions: helium was used as a carrier gas at approximately 1 mL/min, pulsed splitless mode, the solvent delay was 3 min and the injection volume was 1.0 μL. The mass spectrometric detector was operated in electron impact ionization mode operating at 70 eV, ionization energy, scanning from *m/z* 50 to 500. The ion source temperature was 230 °C and the quadruple temperature was 150 °C. The GC temperature program was started at 60 °C then elevated to 280 °C at a rate of 8 °C/min and 10 min and hold at 280 °C for 10 min. The detector and injector temperatures were set at 280 and 250 °C, respectively.

2.4. Identification of the essential oils constituents

Identification of the essential oils constituents was done on the basis of retention time and retention index using a homologous series of *n*-alkanes (C10–C32) under identical experimental conditions, mass spectra library search (Wiley7n.1 and Wiley7 NIST.05.L), and by comparing the mass spectral and retention data with the literature [25]. The relative amounts of individual components were calculated based on the GC peak area.

3. Results

Hydrodestillation of *R. epapposum* flowers, leaves, and stems gave pale yellow oils in yields of 0.5%, 0.1% and 0.000 1% (v/w), respectively based on dry weight. The compounds identified from the different aerial parts of *R. epapposum*, their retention indices and their percentage composition are summarized in Table 1.

A total of 47 constituents, representing 89.91% of the flowers' oil; 34 constituents, representing 94.86% of the leaves' oil and 16 constituents, forming 76.35% of the stems' oil compositions were identified. The oil from the flowers showed limonene (11.75%), linalool (10.10%), 4-terpineol (7.46%), and α-cadinol (5.02%), as major constituents. Moreover, α-terpineol, camphene, β-eudesmol, isobornyl formate, α-pinene, citronellyl acetate, β-myrcene, *trans*-caryophyllene, citronellyl formate, carvacrol, *cis*-pinene hydrate, geraniol, caryophyllene oxide, methyleugenol, γ-terpinene, neryl acetate, bicyclogermacrene, spathulenol, bornyl acetate, and palmitic acid were reported in significant concentrations (1%–5%).

Major constituents in leaves oil were limonene (32.75%), sabinene (13.13%), α-pinene (8.45%) and β-myrcene (7.13%). Other main constituents (1%–5%) were 4-terpineol, β-eudesmol, linalool, γ-terpinene, α-cadinol, *p*-cymene, camphene, α-terpineol, α-terpinene and α-thujene. Stems oil showed the lowest chemical diversity (16 constituents) with linalool