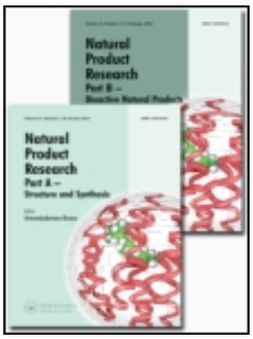

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SHORT COMMUNICATION

Chemical constituents of essential oil of endemic *Rhanterium suaveolens* Desf. growing in Algerian Sahara with antibiofilm, antioxidant and anticholinesterase activities

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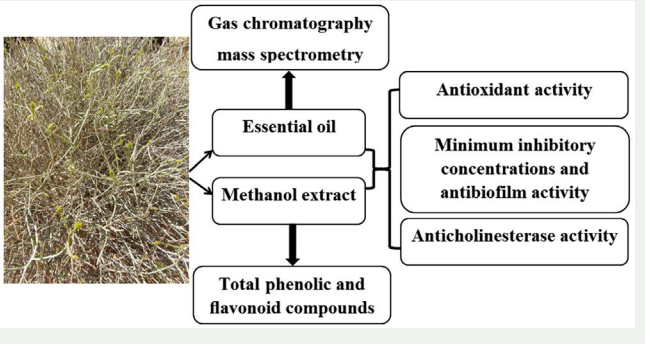
ABSTRACT

Twenty compounds were detected in the essential oil of *Rhanterium suaveolens* representing 98.01% of the total oil content. Perillaldehyde (45.79%), caryophyllene oxide (24.82%) and *β*-cadinol (5.61%) were identified as the main constituents. In β-carotene–linoleic acid assay, both the oil and the methanol extract exhibited good lipid peroxidation inhibition activity, with IC_{50} values of 17.97 ± 5.40 and 11.55 ± 3.39 µg/mL, respectively. In DPPH and CUPRAC assays, however, the methanol extract exhibited a good antioxidant activity. The highest antibiofilm activity has been found 50.30% against *Staphylococcus epidermidis* (MU 30) at 20 µg/mL for essential oil and 58.34% against *Micrococcus luteus* (NRRL B-4375) at 25 mg/mL concentration for methanol extract. The *in vitro* anticholinesterase activity of methanol extract showed a moderate acetylcholinesterase inhibitory (IC_{50} = 168.76 ± 0.62 µg/mL) and good butyrylcholinesterase inhibitory (IC_{50} = 54.79 ± 1.89 µg/mL) activities. The essential oil was inactive against both enzymes.



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1. Introduction

The genus *Rhanterium* is distributed over western North Africa, the Arabian Peninsula, Iraq and Iran. Three species; namely, *R. adpressum* Coss. & Durieu, *R. epapposum* Oliver and *R. suaveolens* Desf. of this genus have been reported in the literature. *R. intermedium* Coss. & Durieu ex Pomel is another species, but it is a hybrid between *R. suaveolens* and *R. adpressum*. *R. suaveolens* Desf., a member of the Asteraceae (Compositae), is locally known as 'Arfadja'. Quézel and Santa reported that it is an endemic desert plant growing in North Africa (Quézel & Santa 1963; Wiklund 1986). Some of the *Rhanterium* species are used in folk medicine as an antidiuretic (Hamia et al. 2013).

There are some studies on the *Rhanterium* species in the literature. In one study, β-eudesmol, 16β-hydroxylupeolyl-3-hexadecanoate, stigmasterol and (+)-3-[3′-(nona-1″-en-3″,5″,7″-trynyl) oxiran-2′-yl] propan-2-ol were isolated from *R. adpressum* (Bouheroum et al. 2007). In another study, scopoletin was isolated from *R. epapposum* (Miana & Al-Hazimi 1983). The composition of *R. adpressum* and *R. epapposum* was also studied (Yaghmai & Kolbadipour 1987; Al-Easa 2004; Al-Mazroa et al. 2006; Kala et al. 2009). Ranthenone glucoside, 9-hydroxylinaloyl glucoside, scopolin, fraxetin, scopoletin and sitosterol-3β-O-[6′-palmitoyl-β-*D*-glucopyranoside] (Oueslati et al. 2007), antimicrobial polyacetyleneic alcohols (Oueslati et al. 2004) and ceramides (Oueslati et al. 2005) were isolated from the *R. suaveolens*.

The essential oil composition of the *R. suaveolens* and biological activities have not been reported earlier, except the antiradical activity using ABTS and DPPH assays (Bouaziz et al. 2009).

This paper is the first study of the chemical composition of essential oil of *R. suaveolens*. Antibiofilm and anticholinesterase activity of essential oil and methanol extract of *R. suaveolens* against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), the chief enzymes of Alzheimer's disease, were also reported for the first time. Hence, the main objectives of this work were: determine the chemical composition of hydrodistillated oil of the aerial parts of *R. suaveolens* using gas chromatography and gas chromatography/mass spectrometry (GC/MS). The antibiofilm effect of sub-MICs of essential oil and methanol extract was assessed by the microplate biofilm assay. The antioxidant capacity of the essential oil and methanol extract was evaluated using three complementary assays; namely, β-carotene-linoleic acid, $DPPH^{\bullet}$ scavenging and CUPRAC assays.

2. Results and discussion

2.1. Essential oil

The EO having yellow colour was obtained from aerial parts using hydrodistillation method and the yield was 0.14% (volume/dry-weight). A total of 20 compounds were identified representing 98.01% of the total oil content. The major compounds were perillaldehyde (45.79%), caryophyllene oxide (24.82%), β-cadinol (5.61%), β-caryophyllene (5.17%) and 8-cedren-13-ol (4.98%). β-pinene (3.21%) and α-irone (1.62%) were also determined as a constituent of the EO (Table S1). Monoterpenoids accounted for 48.25% of the total oil content while sesquiterpenoids amounted to 37.97% of the total oil. The sesquiterpenes and monoterpenes were detected in the ratio of 7.40% and 4.39%, respectively. The EOs of *R. adpressum* and *R. epopposum* were rich in monoterpenoids (Yaghmai & Kolbadipour 1987; Hamia et al. 2013). The EO of *R. suaveolens* also resembled to those oils from classification side.

2.2. Anticholinesterase activity

The anticholinesterase activity of the EO and MeOH extract of *R. suaveolens*, against AChE and BChE enzymes was given in Table S2. Galantamine was the standard drug used for comparison. The MeOH extract exhibited moderate to good inhibitory activity against AChE and BChE enzymes. The IC_{50} values were 168.76 ± 0.62 and 54.79 ± 1.89 µg/mL, respectively. On the contrary, the EO was inactive against both AChE and BChE.

2.3. Total phenolic and total flavonoid contents and antioxidant activity

The total phenolic and total flavonoid contents were performed spectrophotometrically using pyrocatechol and quercetin as standard compounds, respectively. The phenolic content of MeOH extract of *R. suaveolens* was 35.58 ± 0.04 µg pyrocatechol equivalents/g extract and the flavonoid content was 5.5 ± 0.02 µg quercetin equivalents/g extract. It can be said that the *R. suaveolens* is poor in flavonoids. Supportively, in a previous study (Bouaziz et al. 2009), 625 ± 75 mg pyrogallol/100 g and trace milligrams of quercetin/100 g were reported for the same plant collected in November in Douz area in Tunisia. The season and area of collection of the plant may lead to the different results.

Table S3 shows the $DPPH^{\cdot}$ scavenging activity and lipid peroxidation inhibitory activity by β-carotene/linoleic acid assay of the EO and the MeOH extract of *R. suaveolens*. Antioxidant activity of the extract and oil increased dose dependently in both assays. In DPPH assay, the MeOH extract demonstrated good $DPPH^{\cdot}$ scavenging activity (IC_{50} = 0.017 ± 0.004 mg/mL), while the EO exhibited weak activity. In the previous study, the DPPH assay was performed only for MeOH extract and the IC_{50} value was calculated as 1.09 ± 0.19 µg/mL (Bouaziz et al. 2009). These differences may be due to the collection time and locality of the plant.

In lipid peroxidation inhibition assay, the MeOH extract exhibited higher inhibition (IC_{50} = 11.55 ± 3.39 µg/mL) against lipid peroxidation, while the EO exhibited (IC_{50} = 17.97 ± 5.40 µg/mL). The EO composition was given in Table S1. As seen, there are no phenolic compounds to scavenge the DPPH radicals. However, compounds as well as the conjugated mono and sesquiterpenoids are responsible for the lipid peroxidation activity. These compounds can scavenge the singlet oxygen and therefore protect the β-carotene colour against bleaching, indirectly.

Figure S1 shows the results of the CUPRAC (cupric reducing antioxidant capacity) of both the MeOH extract and the EO of *R. suaveolens*. α-Tocopherol and BHT were used as positive controls. As shown in (Figure S1), the activity of the MeOH extract of the *R. suaveolens* was compatible with α-tocopherol at all concentrations. As expected the EO indicated a less reducing activity.

2.4. Determination of MIC's and antibiofilm activity

The MIC and antibiofilm activity results of the EO and methanol extract against six bacteria species and *Candida albicans* are given in Table S4. The oil inhibited the growth of all micro-organisms between 10 and 80 µg/mL concentrations. EO at the MIC's inhibited biofilm formations of all microorganisms tested in various percentages. The oil exhibited the highest antimicrobial activity against *S. epidermidis* MU 30 at 20 µg/mL (MIC/1) and at 10 µg/mL (MIC/2) concentrations with 50.3% and 32.96%, respectively.

According to the results, *B. subtilis* was found to be the most susceptible strain against MeOH extract of *R. suaveolens*. The extract has low activity on the growth of *M. luteus* NRRL B-4375 and *S. epidermidis* MU 30 which were only inhibited at high concentration (25 mg/mL). In the presence of 25 mg/mL extract (MIC), the mean biofilm formation values were equal to 58.34% for *M. luteus* NRRL B-4375 and 49.06% for *S. epidermidis* MU 30.

In the current investigation, the EO exhibited antimicrobial activity, particularly against *S. epidermidis* MU 30, *M. luteus* NRRL B-4375 and *B.* subtilis ATCC 6633. Perillaldehyde, the major compound of the oil is an antimicrobial agent. It demonstrated antimicrobial activity against *B. cereus*, *E. coli* and *S. aureus* (Friedman et al. 2006). Concerning the antimicrobial activity against *B. subtilis*, the oil supported the previous results. However, the oil that showed a weak activity against against *S. aureus*. This may be due to its percentage (45.79%) in the oil which did not reach to the extent necessary to inhibit the growth of those micro-organisms.

3. Conclusion

The anticholinesterase activity against both enzymes was performed for the first time in this study. According to the results the EO indicated no activity. However, the methanol extract exhibited good activity against BChE. Thus, the plant can be used as an anticholinesterase agent, particularly against BChE. However, further studies are necessary to evaluate the origin of the activity. Also, the methanolic extract and the oil proved to be effective antioxidants and antimicrobials in different *in vitro* assays and can be suggested as a natural additive in food and pharmaceutical industries. In the case of antioxidant activities, results obtained from β-carotene/linoleic acid bleaching test were found to be stronger than those obtained from DPPH and CUPRAC systems.

Supplementary material

Experimental details relating to this paper are available online, alongside Tables S1–S4 and Figure S1.

Disclosure statement

No potential conflict of interest was reported by the authors.

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