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RESEARCH ARTICLE

Chemical Composition and Biological Activity of the Essential Oil and Solvent Extracts of *Scaligeria Nodosa*

Amir Reza Jassbi*, Mehdi Zare and Fatemeh Heidary Jamebozorgi

Medicinal and Natural Products Chemistry Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

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Abstract:

Background:

The essential oil and three solvent extracts of the aerial parts of *Scaligeria nodosa* (Boiss.) that were collected in Bamu Mountains (Iran), were screened for their antioxidant, antimicrobial properties and total phenolic contents for the first time.

Methods:

The essential oil was extracted by hydrodistillation and analyzed by capillary gas chromatography-mass spectrometry (GC-MS) and GC flame ionization detector (GC-FID).

Results:

Forty five components were identified in the oil, among them, germacrene D (18.1±0.1%), bicyclogermacrene (1.4±0.0%), and spathunelnol (2.7±0.0%) were the major sesquiterpenoids, *n*-tetradecanol (4.9±0.0%) was the most abundant fatty alcohol and diisobuthyl phthalate (43.9±0.2%) was the most abundant constituent. The extracts of aerial parts of *Sc. nodosa* were prepared using different solvents; dichloromethane (DCM), methanol (MeOH) and MeOH: H_2O (80:20). Antibacterial activity of the oil and each solvent extract was tested against three Gram-positive and three Gram-negative bacteria by nutrient broth micro dilution method. Among the tested microorganisms, *Staphylococcus epidermidis* (IC_{50} 1.25-5mg/ml), *Bacillus subtilis* (IC_{50} 1.25mg/ml) and *Escherichia coli* (IC_{50} 1.25mg/ml) were the most susceptible to the applied oils and the extracts. The antioxidant potential and total phenol contents of the extracts were assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and Folin-Ciocalteu reagent, respectively. Methanol and 80% methanol extracts of the plant showed relatively weak DPPH radical scavenging activity and low amounts of total phenol contents.

Keywords: *Scaligeria nodosa*, *Apiaceae*, Essential oil, Antibacterial, Antioxidant, Gas chromatography.

1. INTRODUCTION

The *Apiaceae* Lindl. or *Umbelliferae* is a family of usually aromatic plants with hollow stems commonly known as umbellifers. The family Apiaceae comprises 300 – 455 genera with 3000 – 3750 species distributed in the northern hemisphere [1, 2]. In the Iranian flora, at least 112 genera including 316 species with about 75 endemic plant species are introduced for the family Apiaceae [3]. The aromatic smell of most species is due to the presence of oleoresin or essential oils in their different organs [4]. Seven *Scaligeria* species are reported in Iran, of which two: *Sc. elata and Sc. nodosa* are endemic [5]. *Sc. nodosa* is distributed in the Fars, Hamadan, Esfahan, Markazi and Lorestan Provinces of Iran [5, 6]. Chemical constituents of the essential oil of *Sc. assyriaca* from Khorasan Province of Iran, were studied by GC-MS and the main components in its leaves and fruits were reported as germacrene D and myristicin, respectively [7]. The chemical compositions of the essential oils (EOs) from different parts of *Sc. tripartita* were analyzed. Geijerene

* Address correspondence to this author at Medicinal and Natural Products Chemistry Research Center, Shahid Abaeiyan Ave., Zip: 71348-53734, Shiraz, Iran, Tel: +98-071-32303872, Fax: +98-071-32332225; E-mails: jassbiar@sums.ac.ir; arjassbi@hotmail.com

was detected as one of the major constituents in the stems, leaves (37%) and fruits (55%), while epoxy pseudoisoeugenol angelate (37%) was reported as the main compound in the roots oil [8]. Nine components were detected in the EO of *Sc. lazica*, among which (*Z*)-*β*-farnesene was the major constituent [9, 10]. Germacrene D (67.9%) was reported as the major constituent of the EO of the aerial parts of *Sc. falcarioides* among 24 compounds [11].

To the best of our knowledge there is no phytochemical or biological activity report on *Sc. nodosa*; therefore, the main aim of this research was to characterize the chemical composition of the EO and the antibacterial activity, total phenol contents and antioxidant activity of the oil and different solvent extracts.

2. MATERIAL AND METHOD

2.1. Plant Samples Collection

Scaligeria nodosa Boiss. was collected in spring 2012 from Bamu Mountain in the North of Shiraz (29°41'46.36" N and 52°41'58.16" E). The plant identification was confirmed by Dr. M. Khosravi at the Department of Biology, University of Shiraz, Iran and a voucher specimen (no. PC-91-1-6.1) was deposited at the herbarium of the Medicinal and Natural Products Chemistry Research Center, Shiraz, Iran. Aerial parts of the plant were air-dried at room temperature (25°C) in the shade.

2.2. Extraction of Essential Oil

The essential oil (0.1% v/w) from 100 g of plant material was extracted for 5 h, using a British Pharmacopeia (BP) hydrodistillation apparatus. The yellowish oil was dried over anhydrous sodium sulfate and stored in a tightly closed dark vial and kept at 4°C until used in the antibacterial tests and GC-MS analyses.

2.3. Solvent Extraction of the Plant

The aerial parts of the plant were extracted by dichloromethane (DCM), methanol (MeOH) and 80% MeOH solvents. Extracts were prepared as follows: 30 g of the dry plant after grinding was macerated in 300 mL of the solvents for 48 h. The extraction was repeated twice and the resulting extracts were combined. The extract was then filtered and concentrated in a rotary evaporator under reduced pressure for the removal of the solvents to obtain residues of DCM (0.350 g; 1.66%), MeOH (1.240 g 4.13%) and 80% MeOH (1.165 g; 3.88% w/w) extracts. The resulting concentrated extracts were kept at -20°C until their use for antimicrobial and antioxidant and total phenol content tests [12].

2.4. Gas Chromatography Analysis

Gas chromatography was performed on an Agilent 6890N chromatograph (Agilent Technologies, USA), with a HP-5 capillary column (30 m × 0.25 mm: 0.25 μm film thickness). The oven temperature was programmed from 60°C to 240°C at 5°C/min and kept 10 min at the final temperature. The carrier gas was N_2 with a flow rate of 0.9 mL/min. Injector and detector (FID) temperature was kept at 240 and 250°C, respectively. The injection volume was 0.4 μL for the oils and -alkanes for calculation of the retention indices.

2.5. Gas Chromatography-Mass Spectroscopy Analyses

The GC-MS was carried out on an Agilent 7890N chromatograph, coupled to an Agilent 5975C mass spectrometer (Agilent Technologies, USA), operating at 70 eV ionization energy, 0.5 s/scan and the mass range: 35-400, equipped with a HP-5MS capillary column (phenyl- (5%) methyl-(95%) siloxane, 30 m × 0.25 mm, film thickness 0.25 μm). Helium was used as the carrier gas at the constant flow of 0.9 ml/min and split ratio of 1:50. The oven temperature was 60°C rising to 250°C at a rate of 5°C /min, then held at 250°C for 10 min; transfer line temperature was set at 250°C.

2.6. Antibacterial Activity

2.6.1. Microorganisms and Media

The microorganisms consisted of three Gram-positive bacteria: *Staphylococcus epidermidis* (PTCC 1114), *Bacillus subtilis* (PTCC 1023), *Staphylococcus aureus* (PTCC1112), and three Gram-negative bacteria: *Escherichia coli* (PTCC1330), *Klebsiella pneumoniae* (PTCC1053) and *Salmonella typhi* (PTCC1609) which were obtained from the

Persian Type Culture Collection (PTCC), Tehran, Iran.

2.6.2. Minimum Inhibitory Concentration (MIC) Using Nutrient Broth Micro-Dilution (NBMD)

Antimicrobial activity tests were performed by the nutrient broth micro-dilution method (NBMD). NBMD was performed by using serial dilution of a stock solution (200 mg/ml) of the essential oil and extracts of *Sc. nodosa* in dimethyl sulfoxide (DMSO). The serial dilutions were performed by the addition of DMSO to reach a final concentration of 0.125-5 mg/ml of the above mentioned tests samples. An aliquot of the samples (5 µL) was added to 95 µl of fresh media followed by 100 µl of the bacterial suspension (OD=0.1 at 600 nm) in a 96-well plate. Chloramphenicol was used as a positive control. The plates were incubated at 37ºC for 24 h in a shaking incubator. Antibacterial activity was detected using a colorimetric method by adding 10 µL of 0.5% INT solution in water in each well at the end of the incubation period for further 30 min. The MIC was defined as the lowest concentration of the test solution that inhibited the growth of the bacteria [13].

2.7. Determination of the Free Radical Scavenging (Antioxidant) Activity

The antioxidant activity of the plant extracts was measured by the method of Blois [14] with some modifications [15] and compared to that for quercetin as a natural standard radical scavenger. The extracts were diluted to different concentrations of 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml and 0.625 mg/ml. 5 µl aliquots of the extract dilutions were mixed with 195 µl of the 100 µM DPPH methanol solution. After 30 min shaking of the solutions in the dark, the absorptions of the DPPH solutions were measured at 517 nm. Quercetin was used as a positive control. Inhibition ratio (percent) was calculated from the following equation: Percentage of DPPH reduction = $((A - A_1) / A) \times 100$), where A is the absorbance of the control, and A_1 is the absorbance in the presence of sample.

The IC_{50}s were calculated by linear regression equations of the DPPH inhibition percentage obtained from different concentrations of the extracts and the standard antioxidants, using Microsoft Excel and Curve Expert statistical programs [12, 15].

2.8. Determination of the Total Phenol Content

The total phenol contents of the DCM, MeOH and 80% MeOH extracts were determined by the Folin-Ciocalteu method as described previously [16]. Briefly, in each micro test well plate, 5 µl of the 10 mg/ml plant extracts or the standard gallic acid solution, 158 µL distilled water and 10 µl Folin-Ciocalteu reagents was added and the solution was shaken briefly on a vortex mixer. After 8.5 min incubation at room temperature, 30 µL of a 0.25% sodium carbonate solution was added to each well. The above solutions were kept in the dark at room temperature for 2 h and the absorbance of the solutions was measured at 765 nm against the blank. The concentrations of the total phenolics were measured against a series of gallic acid standard solutions and expressed as mg equivalent of gallic acid in 1 g dry plant material [16].

2.9. Identification of Compounds

GC-Relative retention indices (RRI) for all constituents were calculated according to Van den Dool method [17], using the retention times of *n*-alkanes (C9-C24) as standards and the essential oil's peaks of the GC chromatograms on a HP-5 column under the same chromatographic conditions. In addition to the GC-RRIs, the compounds were identified by comparison of their mass spectra with the authentic mass spectra [18]. For quantification purposes, relative area percentages obtained by GC-FID were used without the use of correction factors.

3. RESULTS AND DISCUSSION

Hydrodistillation of the aerial parts of *Sc. nodosa* resulted in a yellowish oil in 0.1% (w/w) yield. Analysis of the EO was analyzed by GC-MS and GC-FID identified the main constituents as diisobuthyl phthalate (43.9±0.2%), the sesquiterpenoids; germacrene D (18.1±0.1%), bicyclogermacrene (1.4±0.0%), and spathunelnol (2.7±0.0%); and *n*-tetradecanol (4.9±0.0%) as the most abundant fatty alcohol. The monoterpenoids (1.4%) constituted a small fraction of the oil (Table **1**).

The major constituent of the oil, diisobuthyl phthalate (DBP) and other phthalic acid esters have been reported in several terrestrial plants, microbial strains and marine algae and sponges [19]. The biosynthesis of these types of compounds that are also used as plasticizers and in pharmaceutical paints industries is a matter of several debates [20]. Recently, DBP is reported as a natural metabolite derived from the shikimate pathway and biosynthesized by three

filamentous fungi strains: *Penicillium lanosum*, *Trichoderma asperellum* and *Aspergillus niger.* In addition to isolation from the above mentioned fungal extracts, the crude enzyme activity extracted from the fungi catalyzed the phthalate esters biosynthesis when glucose or protocatechuic acid and butanol were used as the substrates [21]. The phathalic acid esters also suggested as uptake by the living organism, plants, sponges, *etc.* from the environmental pollutions. We have quantified Bis (2-ethylhexyl) phthalate (dioctylphthalate; DOP) in different solvent extracts of *Nepeta laxiflora* collected from the same locality as we collected *Sc. nodosa* and reported petroleum ether as the best extracting solvent. This may exclude the possibility of DOP originating from the solvent impurity [22]. The non-volatile DOP was determined in cow's milk and infant milk formula by HPLC [23]. DBP was detected in volatiles aroma of unprocessed rice [24]. The EOs of seven collections of *Stachys inflata* were reported to contain different amounts of DBP [25]. Didecyl-phthalate was isolated as a broad range antifungal agent from *Burkholderia cepacia;* an environmental bacterial strain. More than 95% of the essential oil of *Leea indica,* a perennial shrub, consisted of phthalic acids esters [26]. The oil has shown moderate antibacterial and antifungal activity [26]. To our knowledge, this is the first report on the composition of the essential oil from *Sc. nodosa*, while the main compounds in the oils of the leaves and fruits of *Sc. assyriaca*, collected from Khorasan province and the aerial parts of the plant *Sc. assyriaca* collected nearby *Sc. nodosa* by us (unpublished data) were reported as undecane (4.7, 0.7, 29.3%), *β*-caryophyllene (13.4, 6.9, 11.1%), α-copaene (10.2, 2.7, 0%), germacrene D (21.1, 13.7, 22.5%), kessan (7.4, 9.5, 0%) and myristicin (6.6, 24.3, 0%), respectively. The presence of sesquiterpenoids, but not monoterpenoids, long chain hydrocarbons or fatty alcohols, aldehyde or phenyl propanoids in different members of the *Scaligeria* species may be chemo taxonomically important but to prove the synthesis of DBP by the plants more experiments must be done [20, 27].

Table 1. Chemical constituents (%) of the essential oil of *Sc. nodosa* analyzed by GC-FID.

Compound	RRI	Area (%)± (SE)[a]
n-heptanal	902	0.11 ± 0.0
α-pinene	930	0.05 ± 0.0
n-heptanol	929	0.2 ± 0.0
β-pinene	974	0.5 ± 0.0
β-myrcene	990	0.3 ± 0.0
n-octanal	1003	0.11 ± 0.0
p-cymene	1022	0.05 ± 0.0
limonene	1025	0.1 ± 0.0
bornyl acetate	1278	0.4 ± 0.0
thymol	1290	0.2 ± 0.0
δ-elemene	1331	0.13 ± 0.0
α-cubenene	1344	0.17 ± 0.0
α-copaene	1368	0.9 ± 0.0
β-bourbonene	1376	0.05 ± 0.0
β-cubebene	1383	0.6 ± 0.0
β-elemene	1388	0.21 ± 0.0
***β*-caryophyllene**[b]	**1409**	**1.2 ± 0.0**
β-copaene	1427	0.24 ± 0.0
6S-2,3,8,8-tetramethyltricyclo[5.2.2.0(1,6)]undec-2-ene	1435	0.24 ± 0.0
α-caryophyllene	1441	0.4 ± 0.0
cis-muurola-4(14), 5-dine	1451	0.9 ± 0.0
***β*-farnesen**	**1459**	**1.2 ± 0.0**
2-dodecenal	1469	0.2 ± 0.0
germacrene D	**1486**	**18.1 ± 0.1**
Bicyclogermacrene	**1487**	**1.4 ± 0.0**
α-muurolene	1490	0.5 ± 0.0
δ-amorphene	1499	0.18 ± 0.0
δ-cadinene gamma	1502	0.5 ± 0.0
Z-γ-bisabolene	1509	0.4 ± 0.0
***δ*-cadinene**	**1517**	**1.2 ± 0.0**
trans-cadina-1(2),4-dine	1524	0.2 ± 0.0
α-cadinene	1529	0.14 ± 0.0

(Table 1) contd.....

Compound	RRI	Area (%)± (SE)[a]
α-calacorene	1534	0.1 ± 0.0
spathunelnol	**1567**	**2.7 ± 0.0**
caryophyllene oxide	**1570**	**1.1 ± 0.0**
salvial-4-(14)-en-1-one	1582	0.95 ± 0.0
β-atlantol	1607	0.5 ± 0.0
1,10-di-epi-cubenol	**1617**	**2.9 ± 0.0**
α-cardinol	1632	0.45 ± 0.0
epi-α-muurolol	1639	0.4 ± 0.0
α-cadinol	1643	0.6 ± 0.0
3-iso-thujopsanone	1648	0.7 ± 0.0
***n*-tetradecanol**	**1674**	**4.9 ± 0.0**
Khusinol	1681	0.7 ± 0.0
diisobutyl phthalate	**1670**	**43.9 ± 0.2**
Total		90.88

[a]The values are the means of three different FID area percentage ± SE.[b]**The major compounds with more than 1.0% area concentration were formatted in bold font.**

The EO showed antibacterial activity against gram positive bacteria *B. subtilis* and *Staph. epidermidis* (IC_{50}=1.25, 5 mg/ ml) and the gram negative *E. coli* (IC_{50}=1.25 mg/ ml) (Table **2**). The methanol extract was active against all of the assayed bacteria (IC_{50}s=1.25-5 mg/ ml) except *K. pneumonia* (Table **2**). *B. subtilis*, *Staph. epidermidis, E. coli, P. aeruginosa* and *K. Pneumonia* were all susceptible to the DCM extracts with IC_{50}=1.25 mg/ml. Finally the weakest plant product was the 80% methanol extract, which only inhibited the growth of *Staph. epidermidis* at IC_{50}=2.5 mg/ ml, indicating a lesser role of possible phenolics or other polar compounds in the antibacterial activity of the plant material. The antioxidant activity and total phenol content of the plant's methanol and 80% methanol extracts were measured using DPPH radical scavenging activity and Folin-Ciocalteu reagents, but the DCM and EO were not active radical scavengers and did not contain detectable amounts of phenolics (Table **3**). The above results indicate that *Sc. nodosa* may be more interesting for investigating antibacterial natural products rather than phenolic antioxidants and also suggest that DCM and MeOH extracts are the best plant extracts for antibacterial activity. The presence of DBP in the oil may be an indicator of natural or environmental polluting source, as they are reported harmful agents to human health.

Table 2. Antimicrobial potential (MIC[a]) of essential oil and solvent extracts from *Sc. nodosa*, as determined by nutrient-broth micro-dilution bioassay.

Plant Extract	*Staph. aureus*	*Staph. epidermidis*	*B. subtilis*	*Sal. typhi*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*
EO	NA	5	1.25	NA	NA	1.25	*NA*
DCM	NA	1.25	1.25	1.25	NA	1.25	*1.25*
MeOH	5	5	1.25	5	5	1.25	*NA*
80% MeOH	NA	2.5	NA	NA	NA	NA	*NA*
Chloramphenicol	0.0125	0.025	0.0125	0.05	NA	0.05	0.05

a) Minimum inhibitory concentration (MIC) the plant extracts in the bacterial suspension in the nutrient broth media (mg/ ml) determined in three replicates. b) NA= not active

Table 3. Total phenolic content and DPPH radical scavenging potential of the methanol extracts from *Sc nodosa*.

Plant Extract	IC50 DPPH[a]	Total Phenol [b]
MeOH	216.47 ± 1.15[c]	16.94 ± 1.47
80% MeOH	195 ± 3.8	38.59 ± 0.66
Quercetin	1.79±0.046	-

a) DPPH IC50 (µg plant extracted or µg quercetin/ 1 ml 10^{-4} M DPPH), b) Total phenol (mg eq. gallic acid in 1g dried plant). The value results are expressed as the means of three different replicates ± SE

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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