Original Article

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Essential oil composition and bioinformatic analysis of Spanish broom (*Spartium junceum* L.)

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Abstract

The essential oil, isolated by hydrodistillation from fresh flowers of *Spartium junceum* L. collected from medicinal plant garden in Shiraz faculty of pharmacy, near Shiraz, Fars, Iran, was investigated by Gas chromatography-Mass spectrometry (GC/MS) method. The GC/MS analysis of the oil revealed the presence of 30 constituents, of which linalool (26.18%), tetradecanoic acid (22.83%), camphor (13.50%), and dodecanoic acid (13.09%) were the major, constituting altogether almost 75.60% of total composition. This is the first report of linalool as a major compound in S. junceum oil composition. For studying of 18S rRNA gene, genomic DNA content was extracted and PCR procedure was done. Sequence similarity searches were done using NCBI database and CLC sequence viewer software. The result of PCR blasted with other sequenced genes in NCBI showed 98% similarity to the 18S small subunit rRNA of *Pisumsativum* (Fabaceae) and Phaseoleae environmental samples of Elev and Amb clones. The phylogenetic relationships among 71 previously reported sequences of ribosomal encoding genes from plants and this novel sequence was investigated as well.

Keywords: Bioinformatics, Essential oil, 18S rRNAencodinggene, Phylogenetic relationships, *Spartium junceum* L.

1. Introduction

Spartium junceum L. (Papilionaceae), Spanish Broom, Gol-e-Tavoosi (in Persian), is indigenous in the Mediterranean countries and cultivated as an ornamental plant in Iran. Spanish Broom is a tall, thornless, deciduous shrub to small tree up to 3 m, with oval leaves sparse 15-25 mm in length, caduceus, flowers 20-30 mm in length and deep golden-yellow, and has terminal and crowded racemes. Fruit is a linear and dehiscent legume with 65-90 mm long, 6-7 mm wide, and 12 to 20 seeds (1).

S. junceum is known for its diuretics, sedative (2), and vasoconstrictor effects (3). The flower infusion is used for treatment of peptic ulcers in southern Anatolia, Turkey (2) and also methanolic extract of *S. junceum* flowers has shown antiulcerogenic effect *in vivo* against ethanol-induced gastric lesions in rats, due to an oleanen-type triterpenicsaponin named spartitrioside (2,4). Other studies have reported antileishmanial activity of extract of branches against *Leishmania major* (5)

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and anti-inflammatory and analgesic activity of hexane extract of flowers in rats (6).

Some flavonoids has been isolated from *S. junceum*, including chrysin, chrysin 7-glucoside, and chrysin 7-gentiobioside from flowers (3); luteolin and genistein (7); apigenin-7-O-β-D-glucopyranoside, genistein-8-C-β-D-glucopyranoside, 5,8-dihydroxy-4´methoxy-6,7-methylenedioxyisoflavone, carthamidin-7-O-α-L-rhamnopyranoside, 3β,16β,22β,24-tetrahydroxy-olean-l2-ene-3-β-yl-[β-D-glucopyranosyl-(l→2)]-α-L-rhamnopyranoside (junceoside) from aerial part(8). Another study indicated that the antiulcerogenic effect of the flowers of *S. junceum* may be because of their high content of flavonoid glycosides, mainly quercetin 3,4´-diglucoside and quercetin 4´β-glucoside, which have antioxidant activity (9). Also, some quinolizidine alkaloids including cytisine, N-methylcytisine, and anagyrine have been extracted from flowers of *S. junceum* (10).

Information about components of the essential oil of *S. junceum* flowers is limited. In a report, tricosane, tetracosane, and pentacosane have been isolated as major components of essential oil derived from fresh flowers of *S. junceum* in South-central Tuscany, Italy (11). In another study, it has been reported that aerial parts of S. junceumin Castelporziano (Italy) emit some volatile organic compounds including isoprene as the major compound;α-thujene, 2-heptanone, camphene, sabinene, β-pinene, myrcene, α-phellandrene, Δ3-carene, α-terpinene, cymene, limonene, γ-terpinene, linalool, α-terpineol , cis-ocimene, 1-phenyl-ethanone , nonanal, and decanal as minor compounds(12).

Molecular markers are used as tools for estimating the phylogenetic relationships of different kinds of organisms (13). Genes encoding ribosomal RNAs and ribosomal proteins have been highly conserved throughout evolution and have diverged more slowly than other chromosomal genes. Comparison of the nucleotide sequence of 18S ribosomal RNA from a range of biologic sources revealed evolutionary relationships among widely divergent organisms (14). Because of the broad spectrum of application of 18S rRNA, using PCR to amplify the complete region of this gene for further analyses will be very useful (13). These gene fragments are commonly used to study phylogenetic relationships between species and could be applied to study several species of plants that present uncertain taxonomic affiliations.

In the present work, the content of essential oilobtained by hydrodistillation method from dried flowers of *S. junceumin* Shiraz, Fars, Iran was studied. The genetic similarity and the conserved domains of this species and four near species of plants were investigated after isolation, identification, and sequencing of 18S rRNA gene. Furthermore, the obtained sequence was aligned with 71 previously reported ribosomal encoding genes from plants and the phylogenetic relationships among them were studied.

2. Materials and methods

2.1. Plant material

S. junceum was collected from medicinal plant garden in Shiraz School of Pharmacy, 5 Km far from Shiraz, Fars, Iran, on May 2006 at its flowering stage. The plant was identified by A. Mohagheghzadeh, Department of Pharmacognosy, School of Pharmacy, Shiraz University of Medical Sciences (SUMS), Iran. Voucher specimens were kept at the herbarium of School of Pharmacy (code: HPSUMS-280), Shiraz University of Medical Sciences. The flowers were dried at room temperature and in shade for 2 weeks and were used for the extraction of essential oil.

2.2. Extraction of the essential oil

The essential oil was obtained by hydrodistillation of 30 g air dried flowers of S. junceum using a Clevenger type apparatus for 4 h. The Essential oil was kept in -20 °C until analysis.

2.3. Gas chromatography-mass spectrometry analysis

The GC-MS analyses were carried out using a Hewlett-Packard 6890. The gas chromatograph was equipped witha HP-5M capillary column (phenyl methyl siloxan, 25 m x 0.25 mm i.d., Hewlett–Packard Part No. 190915.433, USA). The oven temperature was programmed from 50 °C (3 min) to 250 °C at the rate of 3 °C min-1 and fi-

nally held for 10 min at 250 °C. The carrier gas was helium with the flow rate of 1.2 ml.min-1. The mass spectrometer (Hewlett-Packard 5973, USA) was operating in EI mode at70 eV. The interface temperature was 250 °C; mass range was 30-600 m/z. Identification of constituents was based on comparison of the retention times with those of a series of n-alkanestandards (C10 to C30: R8769, Sigma) linear interpolation on computer matching with Willey (275) andspectra literature data (15).

2.4. 18S ribosomal RNA gene sequencing

The sequence of 18S rRNA gene of the selected strain was studied. For this purpose, genomic DNA content was extracted from the petals of HPSUMS-280 and then PCR procedure was applied using two sets of primers.

2.5. DNA extraction

To extract genomic DNA from the pet-

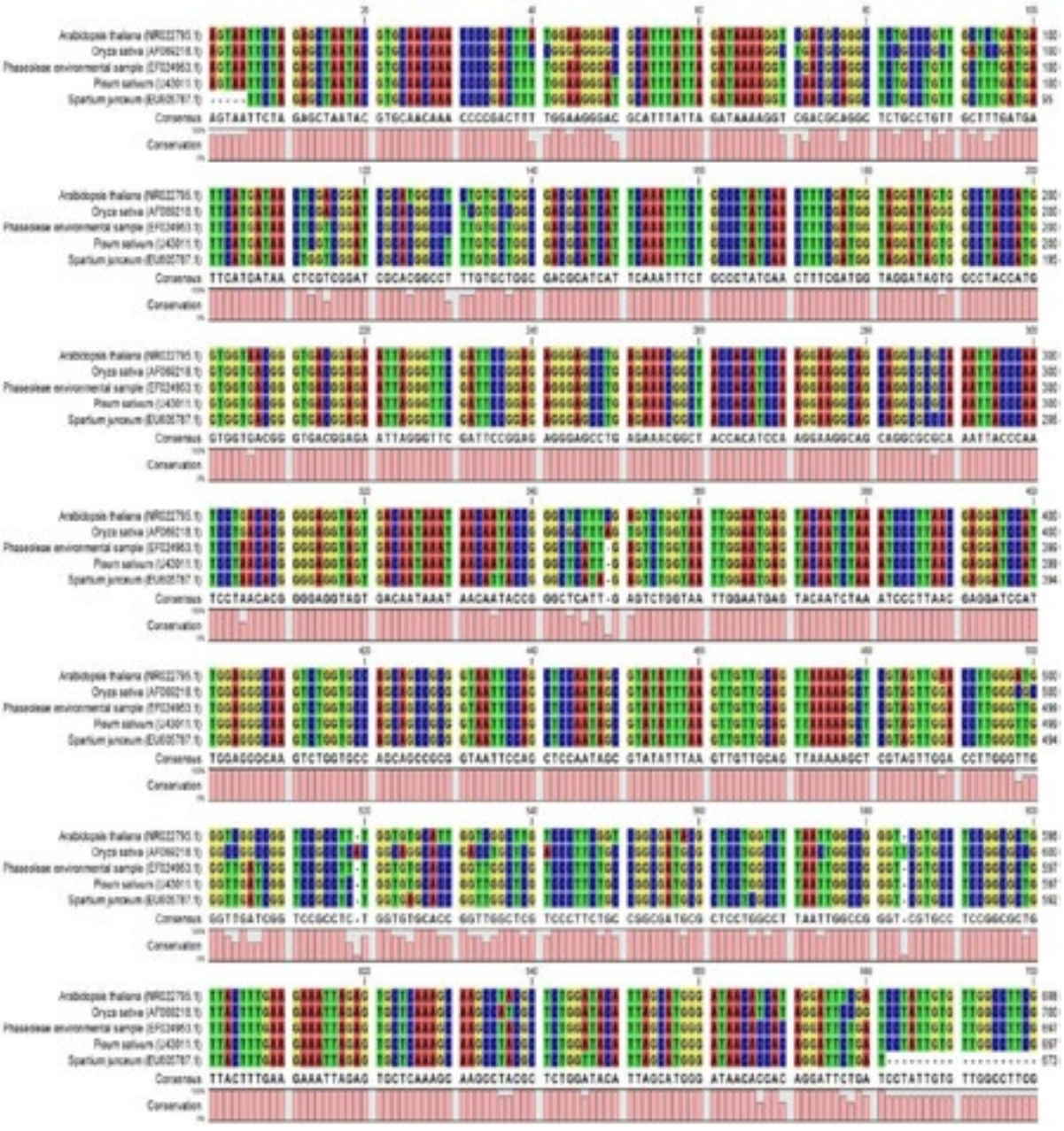

Figure 1. The similarity between amplified gene and other available 18S rRNA genes from NCBI database, the conserved domains (%) are shown by pink color. The accession numbers of each sequence is given in the parentheses.

als ofHPSUMS-280, 200 mg of dried petals was crashed. 400 µl of extraction buffer was added and mixed completely. After centrifugation at 12,000 rpm for 7 min, 300 µl of the supernatant was transferred to a new tube and 300 µl isopropanol was added and mixed completely. Then the resulting solution was centrifuged at 12,000 rpm for 7 min. The supernatant was removed and the pellet was washed with 1 ml ethanol (70 ºC). After removing ethanol, the resulting pellet was dried completely and then 30-50 µl distilled water was added to the pellet and mixed gently. The resulting supernatant was used as template for PCR.

2.6. PCR amplification and sequencing analysis

The two oligonucleotide primers used for amplification of 18S rRNA gene of HPSUMS-280 were 5′-GTCAGAGGTGAAATTCTTGGATT-

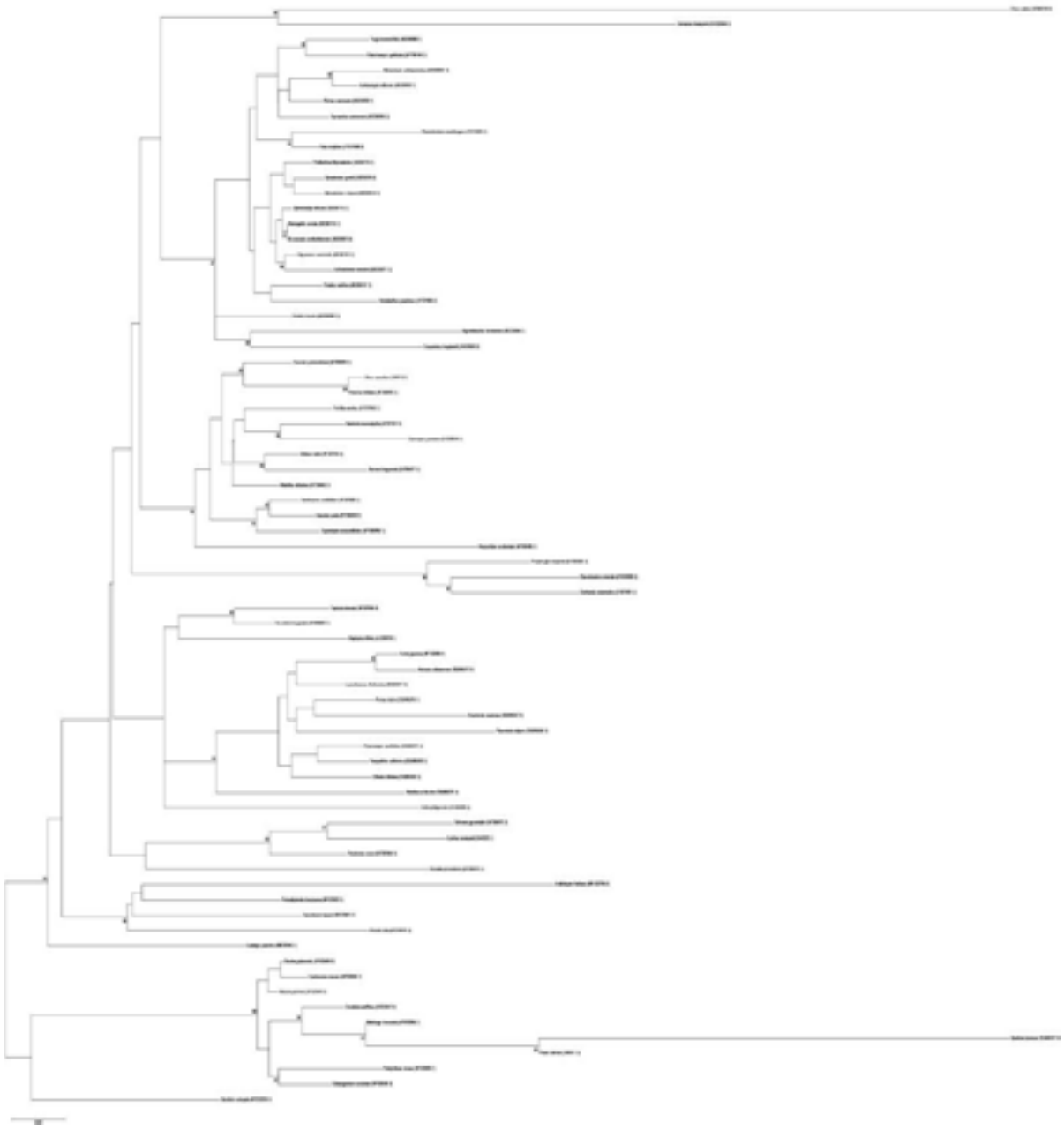

Figure 2. Phylogenetic tree showing the position of *S. junceumamong* 72 species of plants constructed by the Minimum Evolution method on 18S rRNA sequence data of 1819 aligned positions. The bootstrap values less than 50% percent probabilities are not shown. The accession numbers of each sequence is given in the parentheses.

Table 1. List of the components of *Spartiumjunceum* L. flower oil

Compound	% in the oil	KI
Limonene	1.55	1,029
Camphenol	28.67	1,109
trans-Thujone	0.57	1,119
Camphor	14.78	1,148
[unknown]	0.40	1,227
(E)-Cinnamaldehyde	0.24	1,266
[unknown]	0.45	1,291
trans-Caryophyllene	0.31	1,413
[unknown]	2.15	1,495
α-Farensene	1.38	1,506
[unknown]	0.15	1,521
Caryophyllene oxide	1.21	1,577
Ethyl dodecanoate	14.37	1,605
β-Eudesmol	0.58	1,649
[unknown]	0.38	1,698
Tetradecanoic acid, methyl ester	0.49	1,725
Ethyl tetradecanoate	25.00	1,797
[unknown]	0.46	1,846
[unknown]	0.24	1,868
[unknown]	0.30	1,962
Ethyl hexadecanoate	4.14	1,987
Methyl linoleate	0.89	2,098
Tricosane	0.88	2,298
Pentacosane	0.43	2,597

TA-3′ as forward and 5′-AGGGCAGGGACGTA-ATCAACG-3′ as reverse. PCR was performed in a final concentration of 50 µl, containing 5 µl 10X PCR amplification buffer, 0.8 µl Taq DNA polymerase, 1.5 µl of each dNTP, 2 µl of each primer and 15 µl template DNA. Amplification conditions were initial denaturation at 94 ºC for 5 min; 10 cycles at 94 ºC for 30 sec, 50 ºC for 30 sec and 72 ºC for 2 min; 20 cycles at 94 ºC for 30 sec, 50 °C for 30 sec, and 72 ºC for 2.5 min with a final cycle of 72 ºC for 5 min. Taq polymerase was added to the reaction after the first denaturation step. The lower denaturation temperature (92 ºC) during the 20 cycles step was used to avoid loss of enzyme activity (16).

The samples were electrophoresed in a 1% (w/v) agarose gel using TBE buffer containing 1 µg/ml ethidium bromide. A single 700 bp DNA was cut and extracted from the gel using Core Bio Gel Extraction Kit. The sequence was determined by CinnaGen Company with the primers.

The DNA sequence of HPSUMS-280 was recorded in the NCBI under the accession number EU605787. The CLC sequence viewer software, version 5.1.1, a bioinformatic tool for multiple sequence alignment, was used to compare amplified sequence with other 18S rRNA available genes from NCBI database. The conserved domains among these five sequences are shown too (Figure 1).

2.7. The phylogenetic and molecular evolutionary analyses

The obtained sequence was aligned with 71 previously reported sequences of ribosomal encoding genes from plants. For this purpose the MultAlin software, version 5.4.1 was used which creates a multiple sequence alignment from a group of related sequences using progressive pairwise alignments (17). The phylogenetic and mo-

lecular evolutionary analyses were conducted in the MEGA software, version 6.0 (18). The evolutionary history was inferred using the Minimum Evolution method (19).The optimal tree with the sum of branch length=0.38222775 is shown in Figure 2. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test was 1000 replicates (20). The evolutionary distances were computed using the Maximum Composite Likelihood method (21) and are in the units of the number of base substitutions per site. The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm (22) at a search level of 1. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option). There were a total of 1819 positions in the final dataset.

3. Results and discussion

The hydrodistillation of 30 g of dried flowers of S. junceum gave 0.4 ml essential oil (1.33% v/w vs. dried material). The GC-MS analysis of the oil sample showed the presence of 24 compounds which represented 95.49% of the total amount of the oil (Table 1). Among these, camphenol (28.67%), ethyl tetradecanoate (25%), camphor (14.78%), and Ethyl dodecanoate (14.37%) were the major compounds. The monoterpene hydrocarbons constituted 44.02% of the oil. Out of which, camphenol (28.67%) and camphor (14.78%) were the major compounds. The only oxygenated monoterpene was limonene (1.55%). The sesquiterpene hydrocarbons were trans-caryophyllene (0.31%), caryophyllene oxide (1.21%), and beta-eudesmol (0.58%). In Table 1, the oil composition of *S. junceum* L. collected in another climate, in Siena, Italy, as reported by previous work (11) is included for comparative purposes. Although tricosane and pentacosane were the major compounds in the previous work, respectively 8.9% and 16.1%, they were detected in trace.

Moreover, in this study 18S rRNA gene was sequenced and its similarity with other submitted 18S rRNA genes was investigated. The DNA sequence of *S. junceum* L. HPSUMS-280 was recorded in the NCBI. (GenBank accession no. EU605787). It is the first report of determining the partial sequence of a ribosomal encoding gene from the Spartium species. Previously, there was no report about this in the DNA sequence databases. The amplified sequence which had 673 bp nucleotides didn't have 100% homology with any submitted sequences, but it showed 98% similarity with *P. sativum* (Fabaceae) and Phaseoleae environmental samples of Elev and Amb clones. The CLC sequence viewer software, version 5.1.1, was used to compare amplified sequence with other 18S rRNA available genes from NCBI database. The conserved domains are shown, too (Fig. 1). The phylogenetic and evolutionary analyses (Fig. 2) showed a clade of relationship for *S. junceum* and *P. sativum*. The sequence of 18S ribosomal gene from other species of Spartium should be determined to find sister relationships between them.

4. Conclusion

The results of current study revealed the presence of 30 constituents, of which linalool (26.18%), tetradecanoic acid (22.83%), camphor (13.50%), and dodecanoic acid (13.09%) were the major, constituting altogether almost 75.60% of total composition. Although some variations in essential oils may arise from intrinsic and extrinsic conditions such as growth conditions, climatic, environmental, seasonal and distillation processes of the plant samples. The results of current study could provide the background for the detailed studies on the related species of this genus or family as it is the first report on the chemical composition of *S. junceum* essential oil.

5. Acknowledgment

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Conflict of interest

None declared.

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