International Journal of Horticultural Science and Technology (2024) Vol. 11, No. 2, pp. 249-256

International Journal of Horticultural Science and Technology
Journal homepage: http://ijhst.ut.ac.ir

Molecular and Chemical Diversity of *Spartium junceum* in Different Altitudes of Libyan Green Mountains

Khaled Elmeer[1]*, Zenib Suliman[2], Latifa Al-Latifi[3], Nora Alhashimi[3], Ateea Alamami[2]

1 Faculty of Agriculture, University of Tripoli, Libya
2 Omar Mukhtar University, Al-Bayda, Libya
3 Department of Agricultural Research, Ministry of Municipality and Environment, Doha Qatar

ARTICLE INFO

Article history:

Received: 29 May 2023,
Received in revised form: 16 September 2023,
Accepted: 24 September 2023

Article type:

Research paper

Keywords:

Flavonoids,
Genetic diversity,
ISSR,
Phenol,
Spanish broom

ABSTRACT

In the Libyan Green Mountains, this study was conducted to evaluate the effects of different elevations and topography on the genetic variation and chemical composition *of Spartium junceum.* The results indicated a significant difference among the antioxidant activity, phenolic compounds, and flavonoid content of flower extracts obtained from the genotypes of different regions. The total phenol content ranged from 22.74 to 31.66 mg GAE g^{-1}, whereas the flavonoid content ranged from 7.35 to 10.74 mg CE g^{-1} DW. The antioxidant efficiency appeared variable among the flower extracts, ranging between 66.81-91.55 µM Trolox equivalents (TE) g^{-1} of dry matter. Genetic variation was examined by inter-simple sequence repeats, showing a mean value of 0.26 in gene diversity, and a mean value of 0.21 in polymorphism information content. A total of 26 bands were observed, with an average of 3.25 bands per primer, while 50% of the bands were polymorphic. The most effective primers were BT01, BT09, and BT11, which generated four bands. The least effective primer was BT10, which generated only two bands. Phylogenetic relationships between the genotypes divided the samples into two main groups according to geographical location.

Abbreviations: Polymerase chain reaction (PCR), Inter Simple Sequence Repeats (ISSR), Libyan Green Mountain (LGM), Polymorphism Informat ion Content (PIC), Genetic Diversity (GD), 2,2-Diphenyl-1-picrylhydrazy l (DPPH), Tris-acetate-EDTA (TEA), Ultra violet (UV), PAleontological St atistics (PAST), Base Pair (bp), Total Phenols (TP), Total Flavonoids (TF)

Introduction

Spanish broom (*Spartium junceum* L.) is a shrub of the Fabaceae family. It originated in the Mediterranean basin and the Canary Islands (Sanhueza and Zalba, 2012; Tabur et al., 2009). This versatile plant has branches and stems that serve as valuable sources of natural fibers, employed in producing ropes, mats, baskets, and paper. Its flower petals are a source of essential oils, utilized in dyes, and can benefit beekeeping. *Spartium junceum* is a plant with ornamental and medicinal value. It finds application in landscaping, particularly along roadsides and sloping areas, where its deep roots stabilize the soil, fix nitrogen from the atmosphere, and prevent erosion. These actions can enhance soil fertility (Bezic et al., 2003; Katović et al., 2011). The plant exudates serve as a laxative and diuretic. Notably, a significant biochemical compound in *Spartium junceum* is sparteine, which acts as a stimulant for the heart. Traditionally, this compound has been employed in folk medicine to address various concerns, including snakebites and stomach ulcers, and to

*Corresponding author's email: K.Elmeer@uot.edu.ly

stimulate uterine contractions (Nadaf et al., 2012).
The Spanish broom has a broad distribution in the Libyan Green Mountains (LGM), where populations of this species exist in three different altitudes with unique climates. The first altitude is adjacent to the seashore, representing plain lands in a Mediterranean climate. The second altitude level usually has a mild climate throughout different seasons. The third type of terrain, or altitude, has a maximum elevation of 882 meters above sea level and has cold winters and hot summers (El-Barasi and Saaed, 2013). These differences in climate and topography are usually reflected in vegetative characteristics within these areas, resulting in genetic diversity as a crucial precursor of ecological sustainability at different terrain levels of the LGM. The variations in climate and landscape have manifested in vegetative variedness and soil composition across these regions. This environmental heterogeneity significantly determines the composition and abundance of active compounds within medicinal plants. Moreover, it can potentially impact the genetic variability of plants across varying terrain gradients. The genetic diversity within plant species assumes a crucial role in species preservation and adaptive capacity, as emphasized by Reed and Frankham (2003).
In recent times, numerous research investigations have delved into the influence of elevated geographical habitats on the genetic diversity within plant populations. Molecular markers have assisted in gathering pertinent information for such analyses. These studies hold notable significance, given their capacity to assess and elucidate genetic makeup and evolutionary trajectories in populations. Such insights can furnish valuable benchmarks for designing effective conservation strategies (Ohsawa and Ide, 2008).
The challenging conditions inherent to high-altitude environments curtail the growth of plant species due to factors like low temperatures and abbreviated growing seasons, a fact highlighted by Thiel-Egenter et al. (2009).
Despite the Spanish broom's ecological significance, there remains a notable potential for comprehensive exploration in areas like molecular biology, genetic fingerprint identification, and genetic diversity research. Consequently, the present study gears towards assessing the extent of genetic diversity across varying elevations and topographical features, in addition to studying the effect of altitude and different terrains on the chemical compositions of the plant.

Material and Methods

Sample preparation

Fifteen random samples of fresh leaves were collected from three different altitudes. These samples included level 1 (Ras Al-Hilal 39 m), level 2 (Wardamah 430 m), and level 3 (Umm Qudaih 600 m) (Fig. 1). The samples were dried at room temperature and preserved until extraction operations were conducted.

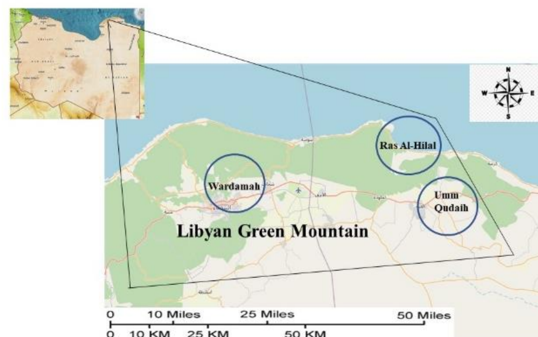

Fig. 1. Different elevation levels in the LGM, i.e., Ras Al-Hilal 39 m, Wardamah 430 m, and Umm Qudaih 600 m above sea level.

Samples of flowers were collected randomly from these areas. The flowers were dried in an oven at 35 ºC (Amer et al., 2013). Then, the samples were ground separately. The samples of each area were mixed to obtain a homogeneous sample. They were placed in sealed, opaque packages and stored at -20 ºC until extraction (Amer et al., 2013).
A methanolic extract of the flowers was made from flower powder (5 g) from each area. Then, 150 ml methanol (80%) was added and placed on a horizontal vibrator (120 cycles min^{-1}) for six hours. The solution was then filtered. The extraction procedure was repeated three times. The methanolic extract was collected and concentrated under vacuum at 35 ºC. After lyophilization, the resultant extract was re-dissolved in methanol (80%) at a concentration of 2.5 mg ml^{-1} and this extract was used for estimating the total amount of phenol, total flavonoids content, and antioxidant activity using the DPPH assay (Amer et al., 2013).
The phenolic content of leaf extract was determined using Folin Ciocalteu according to a modified method described by Habibatni et al. (2016). The total flavonoids were estimated calorimetrically according to a common method (Kosalec et al., 2004). Antioxidant activity was estimated using the DPPH assay, according to Steed and Truong (2008).

DNA extraction

Dried leaf tissues were carefully cleaned with distilled water. One gram of dried leaf tissue was cut into small pieces and ground to a fine powder using liquid nitrogen. DNA was extracted using a pre-prepared plant tissue mixture (DNeasy Plant, Maxi kit) (QIAGEN), based on the manufacturer's instructions in the extraction guide.

Eight ISSR markers were applied to amplify DNA using the genomic DNA of *Spartium junceum* samples (Table 1). AmpliTaq Gold PCR Master Mix was used for preparing the PCR reactions via a Veriti Thermal Cycler (Applied Biosystems).

Table 1. Sequences and annealing temperature of ISSR primers.

ISSR Primer	repeat pattern	Annealing Tm
BT01	(AC)8T	51.4
BT09	(CA)6AC	43.6
BT10	(CA)6GG	46.2
BT11	(CA)6GT	44.7
BT12	(CA)8A	50.3
BT22	(GA)6GG	41.9
BT27	(GT)6CC	46.2
BT28	(GT)6GG	46.6

The resultant mixture (20 µl) comprised 2 µL (20 ng) of DNA, 10 µl AmpliTaq Gold® 360 Mastermix) (manufactured by Applied Biosystems), 1 microliter (5 µmol) of ISSR primer, 7 µL distilled, sterile, nucleic acid-free water. Amplifications were performed using the Hot Start program, involving PCR amplification in a thermal cycler according to the following conditions: a heat-based start at 95 ºC for 10 min, followed by 35 cycles of denaturation at 95 ºC for 30 sec, primer-dependent annealing for 30 sec, extension at 72 ºC for 1 min, and a final extension for 10 min at 72 ºC. The PCR-amplified products of all samples were separated by electrophoresis on 2% agarose gels (Elmeer et al., 2017).

Five microliters of amplified DNA fragments, including a loading dye, were loaded onto the gel (1.5% agarose) at 30 V for 180 min in 1X TAE buffer (30 mmol). The gels were stained with ethidium bromide and visualized on a UV transilluminator. ISSR bands were scored as present (1) or absent (0) to generate a binary.

Statistical analysis

The process involved generating a data matrix by utilizing PAST software version 1.91. This matrix was employed to discern the phylogenetic relationships existing among the genotypes. Subsequently, the phylogenetic diagrams were formulated by the Hamming similarity index as the foundational principle via a method introduced by Hammer et al. (2001).

Results

Extraction yield

With methanol (80%), the extraction yields were 43.09%, 41.60%, and 51.10% from samples of Level 3, Level 2, and Level 1, respectively (Table 2).

Total phenols and flavonoids: phenolic content ranged from 22.74 to 31.66 mg eq. of gallic acid g^{-1} of dry matter (Table 2). The flower extract of samples collected from level 3 showed the highest phenolic content, whereas those of level 2 had the lowest phenolic content. Significant differences were observed among the total phenolic contents in samples collected from the different locations.

Variations were observed in the total flavonoid content of flower extracts from the different locations in the LGM. The flower extract of samples collected from Level 3 showed the highest total flavonoid content (10.74 $\pm$ 0.00), followed by those of Level 1 (9.67 $\pm$ 0.21), while the flower extract of Level 2 showed the lowest value (7.35 $\pm$ 0.21 mg of quercetin eq g^{-1} on a dry weight basis). Significant differences were observed in the total flavonoid content of samples collected from the different locations (Table 2).

The ratio of total phenols to total flavonoids TP/TF%

The results indicated that the total phenolic content of all samples was higher than the total flavonoids (Table 2). The TP/TF ratio was highest in Level 1 (37.74%), followed by Level 3 (33.99%), and the lowest was observed in Level 2 (32.32%).

Antioxidant activity (DPPH assay)

The DPPH assay revealed different levels of antioxidant activity, i.e., 91.55 $\pm$ 0.31, 89.53 $\pm$ 0.08, and 66.81 $\pm$ 0.26 µmol eq. of dry weight, about samples collected from level 3, Level 1, and level 2, respectively (Table 2). The variation was similar to the pattern of values observed in the case of total phenols and total flavonoids.

According to correlation analysis, the total phenolic content correlated significantly and positively with the total flavonoid content ($r = 0.907$; $P \leq 0.001$). Also, the total phenolic content correlated significantly and positively with DPPH values ($r = 0.797$; $P \leq 0.01$). Similarly, but more significantly, the total flavonoid content correlated positively with DPPH values ($r = 0.959$; $P \leq 0.001$).

Table 2. Phenols, flavonoids, and DPPH values in the flower extracts of *Spartium junceum* in various locations of the LGM.

Location	Yield %	Total phenols (mg GAE g^{-1} dw)	Total flavonoids (mg QE g^{-1} dw)	TP/TF Ratio (%)	DPPH (μM TE g^{-1} dw)
Umm Qudaih	43.09a ± 3.72	31.60a ± 0.16	10.74a ± 0.00	33.99	91.55a ± 0.31
Ras Al-Hilal	51.10a ± 1.93	25.62b ± 0.09	9.67b ± 0.21	37.74	89.53b ± 0.08
Wardamah	41.60a ± 0.38	22.74c ± 0.11	7.35c ± 0.21	32.32	66.81c ± 0.26

Correlations

Total phenolic content correlated significantly and positively with the total flavonoid content ($r = 0.907$; $P \leq 0.001$). Also, the total phenolic content correlated significantly and positively with DPPH ($r = 0.797$; $P \leq 0.01$). Similarly, but more significantly, the total flavonoid content correlated positively with DPPH values ($r = 0.959$; $P \leq 0.001$) (Table 3).

Table 3. Correlation coefficient between phenolic content and antioxidant activity.

	TP	TF	DPPH
TP	1		
TF	0.906799	1	
DPPH	0.797012	0.959038	1

Genetic diversity

The electrophoresis of PCR products showed that *Spartium junceum* produced twenty-six bands, with an average of 3.25 bands per primer, using the ISSR technique. A maximum number of four bands were observed with primers BT01, BT09, and BT11, whereas a minimum of two bands was observed with primer BT10 (Table 4). The results indicated that 50% of the bands were polymorphic (Table 4). The amount of polymorphism varied and depended on the primer. Polymorphism was 100% with primers BT10 and BT28 but was 25% with primer BT09. Meanwhile, primer BT09 showed four bands that ranged from 250 to 800 bp (Fig. 2).

Table 4. Amplified DNA bands and polymorphism percent of ISSR primers.

No.	Primer	Bands no.	Polymorphic	(%)
1	BT 01	4	2	50%
2	BT 09	4	1	25%
3	BT 10	2	2	100%
4	BT 11	4	2	50%
5	BT 12	3	1	33%
6	BT 22	3	0	0%
7	BT 27	3	2	66%
8	BT 28	3	3	100%
Total		26	13	
Average		3.25		**50%**

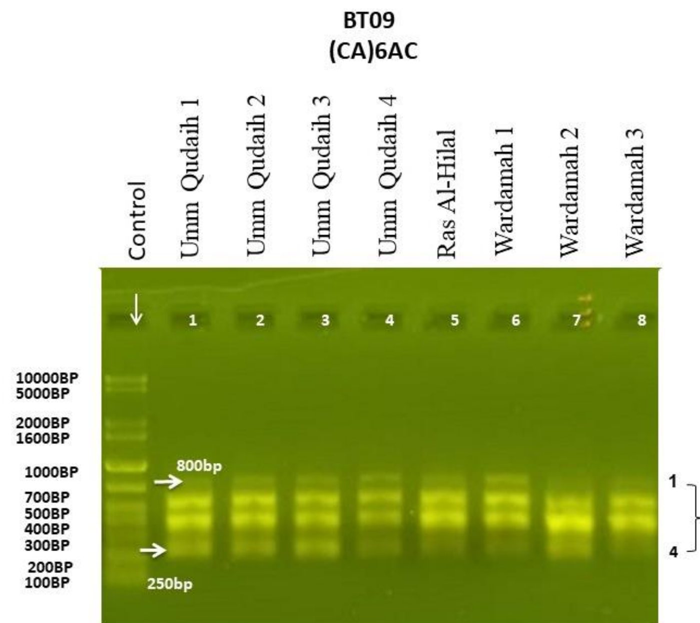

Fig. 2. BT09 ISSR marker patterns of *Spartium junceum* grown in different elevations of the LGM.

The genetic diversity of *Spartium junceum* was 26%. *Spartium junceum* populations were classified into two primary groups through analysis of ISSR markers using a dendrogram. The initial group encompassed plants exclusively from the Level 2 area, whereas the subsequent group comprised some plants in Level 3 and Level 1 (refer to Fig. 3). The most minimal genetic similarity (58%) was noted between the second sample in Level 2 and a sample in Level 1.

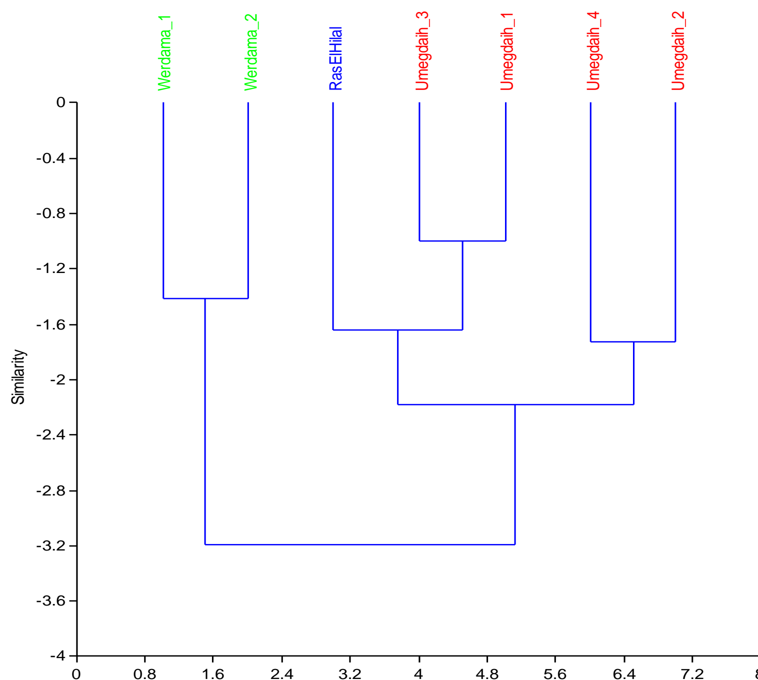

Fig. 3. Dendrogram cluster derived from ISSR markers of *Spartium junceum* plants grown in different altitudes of the LGM.

Discussion

The results indicated no significant differences between the samples in terms of yield, although yield was generally a dependent variable of the amount and type of raw materials and solvent, as well as the extraction method (Goli et al., 2005). In the current research, the phenolic content was higher than 4.8 mg gallic acid g^{-1} dry matter, which agrees with a previous study on a group of aromatic plants in Greece (Proestos et al., 2006). The phenolic content was also higher than two methanolic extracts obtained from the stalks of *Spartium junceum* in Algeria (Habibatni et al., 2016; Wafa, 2019), which showed values of 12.52 and 8.6 mg gallic acid eq g^{-1} dry matter, respectively. The remarkable difference in the total content of extracts between our study and other studies is attributable to environmental factors, genetic differences, sample selection, sampling time, and extraction methods (Bouayed et al., 2007; Shan et al., 2005). Flavonoids are secondary metabolites in plants that produce yellow pigments and play a significant role in color determination. As the most numerous phenols, they are found throughout the plant kingdom and exhibit anti-inflammatory, anti-allergic, and anti-cancer effects (Kang et al., 2010).

The current results indicated that the total flavonoid content (10.74 ± 0.00) was higher than that observed in a previous measurement (8.27 ± 1.14 mg eq. quercetin g^{-1} of extract) reported by Wafa (2019). It was also higher than the total flavonoid content of an alcoholic extract (50% ethanol) and an aqueous extract of *Spartium junceum* flowers in Italy, with values of 0.096 ± 0.002 and 0.02 ± 0.002 g quercetin g^{-1} of fresh weight, respectively.

The DPPH assay is a widely accepted method for evaluating free radical scavenging activity (Naik et al., 2003), where the free radical scavenging ability of antioxidants through DPPH is attributable to its hydrogen donating ability (Liu et al., 2013). The values of the DPPH assay (Table 2) were 91.55 ± 0.31, 89.53 ± 0.08, and 66.81 ± 0.26 μmol eq. of the dry sample, belonging to Level 3, Level 1, and Level 2, respectively, which established a similar pattern observed in the case of total phenolic content and total flavonoids.

The results of correlation analysis were consistent with previous findings that indicated a highly significant correlation ($r = 0.98$, $P \leq 0.01$) between total phenolic content and antioxidant activity (Su and Chien, 2007). It also agreed with previous reports by Liu et al. (2008), showing a significant correlation between the total phenolic content in fruits of *Phyllanthus emblica* L. and their antioxidant activity. A strong correlation was observed between phenolic content and DPPH values ($r = 0.972$) (Anastasiadi et al., 2010), where accurate estimations of the antioxidant properties occurred by considering their total phenolic content. A study on *Cytisus scoparius* in Portugal showed a positive correlation between antioxidant activity and phenolic content (Luis et al., 2009).

Compared to the current results, where twenty-six bands were observed with an average of 3.25 bands per primer, a previous study on chickpeas revealed 21.4 bands with an average of 1.64 bands per primer, using seven ISSR primers (Gautam et al., 2016). In contrast, 132 bands with an average of 4.6 bands per primer were achieved by 29 ISSR primers in date palms (Elmeer et al., 2017). Band count variations can be explained by differences in plant genotype and previous primer-related studies.

In the current study, polymorphism varied per primer and reached 100% with primers BT10 and BT28, whereas polymorphism was 25% in primer BT09. The results are comparable to those obtained by Nautiyal and Panwar (2016) in their study on the evaluation of genetic diversity in chickpeas using seven primers of the ISSR marker, where polymorphism ranged from 50 to 100%. In Iran, 17 *Hypericum* species were evaluated by genomic DNA amplifications using 10 ISSR primers, producing 141 bands, of which 127 were polymorphic (95.78%) (Ma et al., 2021). A high average value of PIC revealed the strong ability of ISSR primers to detect polymorphic loci (Ma et al., 2021). Here, primer BT09 showed four bands that ranged from 250 to 800 bp (Fig. 2). Elmeer and Almalki (2011) reported seven polymorphic bands in Prosopis species, where more than nineteen bands were obtained in twenty-seven genotypes of strawberry with molecular weights that ranged from 400 to 3800 bp (Debnath et al., 2008). In total, 212 repeatable amplified bands were generated in 802 landraces of *Vicia faba* L. from different geographical locations of China and abroad using 11 ISSR primers, of which 209 were polymorphic (Wang et al., 2012).

In a relevant study, the genetic diversity of *Spartium junceum* was reportedly 26% (Liu and Muse, 2005), which is lower than 77% in wild soybean species (Li et al., 2010). Genetic diversity in Napier grass (*Pennisetum purpureum*) was 54%, and PIC was 50%, which is a moderate percentage in the usual capacity of farmers and vegetative propagation. Thus, it reduces the likelihood of new genotypes occurring from cross-pollination (Kawube et al., 2015).

The lowest degree of genetic similarity indicated limited gene flow or ancestrally shared alleles

between plants of different altitudes. However, the most significant values in genetic similarity were 97%, between the first and third samples of plants growing in the Level 3 region. Genetic similarities among seventeen collections of *Hypericum* species from Iran ranged between 0.617 and 0.911 (Ma et al., 2021). The genetic relationship of plants in germplasm was reportedly associated with their geographical origin and ecological habit (Wang et al., 2012).

Conclusion

The findings revealed notable distinctions among various extracts of *Spartium junceum* flowers regarding their phenolic compounds, flavonoids, and antioxidant activity. Elevated altitude regions exhibited the most substantial overall proportion of phenols, flavonoids, and antioxidants. Arrangements in the phylogenetic tree segregated the samples into two principal clusters, suggesting a marginal correlation between genetic relatedness and the geographical positioning of the surveyed areas. We recommend further research on this species in Libya to build up insights into the genetic diversity of *Spartium junceum* in the local gene pool, focusing on resource preservation. Additionally, thoroughly exploring the potential utilization of flower biochemical compounds as biocides against agricultural pests would be beneficial.

Acknowledgements

The authors are grateful to Omar AlMukhtar University Bayda Libya for providing the facilities to complete this work.

Contributions

KE performed the experimental part of the work, wrote the manuscript, interpreted the data, and helped in some experimental work. ZS collected the samples, performed part of the experimental work, followed up the study, and provided assistance throughout the work. LA and NA performed the practical part of the work. AA supervised and implemented the chemical part. All authors read and confirmed the final manuscript.

Conflict of Interest

The authors indicate no conflict of interest in this work.

References

Amer A, Zaeid M, Al-Mabrouk AH. 2013. Effects of twenty-eight plant extracts as insecticides against adults of the sweet potato whitefly *Bemisia tabaci Gennadius* (*Homoptera: Aleyrodidae*) on tomato plants. In International Conference on Applied Life Sciences, UAE.

Anastasiadi M, Pratsinis H, Kletsas D, Skaltsounis AL, Haroutounian SA. 2010. Bioactive non-coloured polyphenols content of grapes, wines and vinification by-products: evaluation of the antioxidant activities of their extracts. Food Research International 43, 805-813.

Bezic NA, Dunkic VA, Radonic A. 2003. Anatomical and chemical adaptation of *Spartium junceum* L. in arid habitat. Acta Biologica Cracoviensia Series Botanica 45, 43-47.

Bouayed J, Piri K, Rammal H, Dicko A, Desor F, Younos C, Soulimani R. 2007. Comparative evaluation of the antioxidant potential of some Iranian medicinal plants. Food Chemistry 104, 364-368.

Debnath SC, Khanizadeh S, Jamieson AR, Kempler C. 2008. Inter simple sequence repeat (ISSR) markers to assess genetic diversity and relatedness within strawberry genotypes. Canadian Journal of Plant Science 88, 313-322.

El-Barasi YM, Saaed MW. 2013. Threats to plant diversity in the north eastern part of Libya (El-Jabal El-Akahdar and Marmarica Plateau). Journal of Environmental Science and Engineering. 1;2,41.

Elmeer K, Almalki A. 2011. DNA finger printing of *Prosopis cineraria* and *Prosopis juliflora* using ISSR and RAPD techniques. American Journal of Plant Sciences 2, 427-534.

Elmeer K, Alghanem M, Al-Latifi L, Alhemairi H. 2017. Efficiency of RAPD and ISSR markers for the detection of polymorphisms and genetic relationships in date palm. Biotechnology 16, 19-26.

Gautam AK, Gupta N, Bhadkariya R, Srivastava N, Bhagyawant SS. 2016. Genetic diversity analysis in chickpea employing ISSR markers. Agrotechnology 5, 2168-9881.

Goli AH, Barzegar M, Sahari MA. 2005. Antioxidant activity and total phenolic compounds of pistachio (*Pistachia* vera) hull extracts. Food chemistry 92, 521-525.

Habibatni S, Miceli N, Ginestra G, Maameri Z, Bisignano C, Cacciola F, Utczas M, Mondello L, Anwar S, Benayache S, Atrouz D. 2016. Antioxidant and antibacterial activity of extract and phases from stems of *Spartium junceum* L. growing in Algeria. International Journal of Phytomedicine 8, 37-46.

Hammer Ø, Harper DA. 2001. Past: paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4-9.

Kang J, Li Z, Wu T, Jensen GS, Schauss AG, Wu X. 2010. Antioxidant capacities of flavonoid compounds isolated from acai pulp (*Euterpe oleracea* Mart.). Food Chemistry 122, 610-617.

Katović D, Katović A, Antonović A. 2011. Extraction methods of Spanish broom (*Spartium Junceum* L.). Wood Industry/Drvna Industrija 62, 255-261.

Kawube G, Kabirizi J, Namazzi C. 2015. Genetic characterization of *Alupe napier* grass accessions based on simple sequence repeat markers. American Journal of Experimental Agriculture 7, 205-213.

Kosalec I, Bakmaz M, Pepeljnjak S, Vladimir-Knezevic SA. 2004. Quantitative analysis of the flavonoids in raw propolis from northern Croatia. Acta Pharmaceutica 54, 65-72.

Li YH, Li W, Zhang C, Yang L, Chang RZ, Gaut BS, Qiu LJ. 2010. Genetic diversity in domesticated soybean (*Glycine max*) and its wild progenitor (*Glycine soja*) for simple sequence repeat and single-nucleotide polymorphism loci. New Phytologist 188, 242-253.

Liu J, Jia L, Kan J, Jin CH. 2013. In vitro and in vivo antioxidant activity of ethanolic extract of white button mushroom (*Agaricus bisporus*). Food and Chemical Toxicology 51, 310-316.

Liu K, Muse SV. 2005. PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21, 2128-2129.

Liu X, Zhao M, Wang J, Yang B, Jiang Y. 2008. Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six regions in China. Journal of Food Composition and Analysis 21, 219-228.

Luis A, Domingues F, Gil C, Duarte AP. 2009. Antioxidant activity of extracts of Portuguese shrubs: *Pterospartum tridentatum, Cytisus scoparius* and *Erica* spp. Journal of Medicinal Plants Research 3, 886-893.

Ma S, Khayatnezhad M, Minaeifar AA. 2021. Genetic diversity and relationships among *Hypericum* L. species by ISSR markers: a high value medicinal plant from northern of Iran. Caryologia 74, 97-107.

Nadaf M, Halimi M, Mortazavi M. 2012. Identification of nonpolar chemical composition *Spartium junceum* flower growing in Iran by GC-MS. Middle-East Journal of Scientific Research 11, 221-224.

Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohoni DP, Biyani MK, Mohan H. 2003. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. Phytochemistry 63, 97-104.

Ohsawa T, Ide Y. 2008. Global patterns of genetic variation in plant species along vertical and horizontal gradients on mountains. Global Ecology and Biogeography 17, 152-163.

Proestos C, Boziaris IS, Nychas GJ, Komaitis M. 2006. Analysis of flavonoids and phenolic acids in Greek aromatic plants: investigation of their antioxidant capacity and antimicrobial activity. Food Chemistry 95, 664-671.

Reed DH, Frankham R. 2003. Correlation between fitness and genetic diversity. Conservation Biology 17, 230-237.

Sanhueza C, Zalba SM. 2012. Experimental control of Spanish broom (*Spartium junceum*) invading natural grasslands. Management of Biological Invasions 3, 97-104.

Shan B, Cai YZ, Sun M, Corke H. 2005. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. Journal of Agricultural and Food Chemistry 53, 7749-7759.

Steed LE, Truong VD. 2008. Anthocyanin content, antioxidant activity, and selected physical properties of flowable purple-fleshed sweet potato purees. Journal of Food Science 73, 215-221.

Su MS, Chien PJ. 2007. Antioxidant activity, anthocyanins, and phenolics of rabbiteye blueberry (*Vaccinium ashei*) fluid products as affected by fermentation. Food Chemistry 104, 182-187.

Tabur S, Cesur A, Özkul H. 2009. Karyology of *seven Fabaceae* taxa from Turkey. Journal of Applied Biological Sciences 3, 49-53.

Thiel-Egenter C, Gugerli F, Alvarez N, Brodbeck S, Cieślak E, Colli L, Englisch T, Gaudeul M, Gielly L, Korbecka G, Negrini R. 2009. Effects of species traits on the genetic diversity of high-mountain plants: a multi-species study across the Alps and the Carpathians. Global Ecology and Biogeography 18, 78-87.

Wafa N. 2019. Antioxidant and anti-inflammatory activities valorisation of methanol extract of two Fabaceae (*Genesta pseudo-pilosa* and *Spartium junceum* L) growth in East of Algeria. International Journal of Chemistry and Pharmaceutical Sciences 7, 60-63.

Wang HF, Zong XX, Guan JP, Yang T, Sun XL, Ma Y, Redden R. 2012. Genetic diversity and relationship of global faba bean (*Vicia faba* L.) germplasm revealed by ISSR markers. Theoretical and Applied Genetics 124, 789-797.

COPYRIGHTS ©2021 The author(s). This is an open access article distributed under the terms of the Creative Commons Attribution (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, as long as the original authors and source are cited. No permission is required from the authors or the publishers

CC BY