ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
https://www.icf.ro/rrch/

Rev. Roum. Chim.,
2025, *70*(1–2), 43–52
DOI: 10.33224/rrch.2025.70.1-2.04

PISTACIA LENTISCUS L., *PHILLYREA LATIFOLIA* L., *GLOBULARIA ALYPUM* L. AND *CERATONIA SILIQUA* L.: UPLC-PDA-MS-tQ ANALYSIS OF THE PHENOLIC CONTENT LEAVES' IN RELATION TO THEIR GROWING AREA

Sana DALLALI,[a,b] Hajer RIGUENE,[c] Sondes FKIRI,[a,d] Lamjed TOUMI,[b] Ramon CANELA–GARAYOA,[e] Ridha Ben SALEM,[c] Ghayth RIGANE,[c,f] Hanene GHAZGHAZI[a] and Jordi ERAS[e,g]

[a] Laboratory of Management and Valorization of Forest Resources (LR16INRGREF01) National Research Institute of Rural Engineering (INRGREF), Water and Forests, University of Carthage, Ariana 2080, Tunis, Tunisia
[b] Silvo-Pastoral Institute of Tabarka, University of Jendouba, Tunisia
[c] Laboratory of Organic Chemistry (LR17ES/08), Faculty of Sciences of Sfax, University of Sfax, BP 1171, 3038, Sfax, Tunisia
[d] Higher Institute of Social Sciences and Education of Gafsa (ISSSEG), Route de Tozeur - 2112 Gafsa, University of Gafsa, Tunisia
[e] Scientific Technical Service TCEM, University of Lleida, Avda. Alcalde Rovira Roure, 191, E-25198 Lleida Spain
[f] Chemistry-Physics Department, Faculty of Sciences and Technology of Sidi Bouzid, B. P 380, 9100, Sidi Bouzid, University of Kairouan, Tunisia
[g] DBAR+D Tecnio Center, Chemistry Department, University of Lleida, Avda. Alcalde Rovira Roure, 191,E-25198 Lleida, Spain

Received August 21, 2024

The chemical composition of plants varies according many factors (climatic and environmental factors, geographical area, drought and diseases, period of sample collection and stage of growth and development of the plant) within the same plant. The goal of this study is to determine the chemical composition of four plants (*Pistacia lentiscus* L., *Phyllaria latifolia* L.,*Globularia alypum* L. and, *Ceratonia silique* L.) via UPLC-PDA-MS/MS-tQ analysis, and evaluating their total phenolic content (TPC), total flavonoid content (TFC) in relation to their growing area. The results revealed that the highest total phenol and flavonoid contents have been found in *G. alypum* (7.92 mg GAE/g DW) and *P. latifolia* (8.31 mg QE/g DW), respectively compared with other extracts in the plot of site 3 (latitude 36°21′88,893″ (N); longitude 10°06′22,593″ (E); altitude 850 m). The results of UPLC-PDA-MS/MS-tQ analysis showed that the main compounds found in all plants were digalloylquinic, trigalloylquinic acids, verbascoside, myricetin-rhamnoside, apigenin-diglycoside, luteolin-hexose and quercetin-3-*O*-rutinoside. These results are the first study highlighted that *Pistacia lentiscus* L., *Phillyrea latifolia* L., *Globularia alypum* L. and *Ceratonia siliqua* L. leaves are an excellent phenolic compounds related to their growing area which could be exploited in the pharmaceutical and food sectors.

INTRODUCTION

Since antiquity, medicinal and aromatic plants have been used in many different fields such as pharmaceutical, medical, industries.[1] Natural herbal remedies have been used for centuries to treat diseases in several civilizations worldwide.[2] One of the major originalities of plants is related to their ability to produce very diverse natural substances. Indeed, alongside the classic primary metabolites (carbohydrates, proteins, lipids, nucleic acids), they frequently accumulate so-called secondary

* Corresponding author: gaith.rigane@yahoo.fr; Phone : +216 97 23 40 87

metabolites, whose physiological function is not always obvious. However, these secondary metabolites represent an important source of molecules that can be used by humans in various fields, such as pharmacology or the food industry.[3] Among these molecules, phenolic compounds are widely distributed and are the most abundant secondary metabolites in plants.[4] It has been suggested that the possible health benefits, of consuming these metabolites, derive from their antioxidant and anti-inflammatory properties.[5] Tunisia has a rich and varied flora in medicinal and aromatic plants, most of which are a spontaneous. Exploring the benefits of these plants remains a field with a major importance to the country. The present work fits in this framework and aims to evaluate the content of total phenols and total flavonoids of the methanolic extract, of the leaves of four plants species: *Pistacia lentiscus*, is an important source of active substances; indeed, several parts of this plant (fruits, bark and leaves), have been used in traditional medicine since Greek civilization.[6] Many studies show that *Phillyrea latifolia L.*, which is close to the olive tree, has a strong therapeutic potential. The study of the composition of the leaves of *Globularia alypum*L.is important in order to confirm the role, mentioned in the literature, that this species plays in the prevention of chronic diseases such as heart pathologies, cancer, diabetes, hypertension, 99 and Alzheimer's disease by combating oxidative stress. On the other hand, *Ceratonia siliqua* L., is a tree with important ecological, industrial and ornamental interests. This species is known for its medicinal and therapeutic properties, such as the pharmacological activities of its phytochemicals. In Tunisia, these four species belong to the forest domain. The main goal of this study is to contribute their development in order to encourage their protection further. A comparison, with previous studies, under other climatic conditions, would make possible to identify the environmental effect on their phytochemical composition. The effect of environmental degradation on the yield, of these four species, in secondary metabolites will be, also, evaluated, by choosing plots with different levels of degradation.

MATERIALS AND METHODS

Plant collection

Leaves of four plant species (*Pistacia lentiscus* L., *Phillyrea latifolia* L., *Globularia alypum* L. and *Ceratonia siliqua* L.), were collected from 3-sites, in the "Park of Djebel-Zaghouan", located in the northeast of Tunisia. Site 1 [latitude 36°21′95,056″ (N); longitude10°05′43,773″ (E); altitude 639 m]; (2) Site 2 [latitude36°21′80,900″ (N); longitude 10°05′66,149″ (E); altitude 656 m]; Site 3 [latitude 36°21′88,893″ (N); longitude 10°06′22,593″ (E); altitude 850 m]. These sites belong to the sub-humid bioclimatic zone, with an average annual rainfall of 550 mm. The harvested materials were air-dried, at room temperature, for one week, then they were ground in a Retch mixer grinder (Normandie-Labo, Normandy,France) and sieved through 0.5 mm mesh screen to obtain an uniform particles size.

Chemicals

Preparation of methanolic Extracts

The extraction method of Total phenolic content (TPC) and Total flavonoids content (TFC) were conducted as follow: three replicates of 1g of each sample were extracted by shaking, with 20 ml of absolute methanol, at room temperature for 30 min. The extracts were stored for 24 h at +4° C, then filtered on Whatman filter paper and evaporated under vacuum in dryness and stored at + 4°C until analysis. For the UPLC analysis of polyphenols, the extraction method has been done according as described previously by Delpino-Rius, *et al.*[7,8] Samples for the UPLC analysis: 2 g of each extract was centrifuged at 30,400x g (Hettich Eppendorf Centrifuge MIKRO 22 R; Germany). Then 0.5 mL of the supernatant was diluted 1:2 with Milli-Q water (acidified at 0.1% v/v with acetic acid) and filtered using a 0.22 µm PTFE filter. Solutions were kept at 6 °C until UPLC analysis. All solvents contained ascorbic acid (0.2% w/v).

Total phenolic content

The total phenolic compounds were assayed according to the method of Fkiri *et al.*[9] with slightly modifications.

Total flavonoids content

The flavonoid content of the extracts of the plants studied was determined as reported previously by Fkiri *et al.*[9]

UPLC-PDA-MS-tQ parameters

Ultra-performance liquid chromatographic analysis was performed on a Waters ACQUITY UPLC-system (Waters, Milford, MA, USA) consisting of an ACQUITY UPLC – binary solvent manager and ACQUITY UPLC™ sample manager,

coupled to a photodiode array detector ACQUITY UPLC™ PDA and fluorescence detector ACQUITY UPLC™ FLR. Compounds were separated with an ACQUITY UPLC™ HSS T3 column (1.8 µm; 2.1 mm × 150 mm) (Waters, Manchester, UK) using a mobile phase consisting of solvent A, H_2O (0.1% v/v HAcO), and solvent B, ACN 100% (0.1% v/v HAcO). The flow rate was 0.55 mL/min. The linear gradient was as follows: 0–1.89 min, 1% B, (isocratic); 1.89–17.84 min, 30% B, (linear gradient); 17.84–21.39 min, 5% B, (linear gradient); 21.39–21.56 min, 1% B, (linear gradient); and 21.56–25 min, 1% B, (isocratic). Weak and strong needle solvents were H_2O (0.1% v/v HAcO) and MeOH, respectively. The injection volume was 20 µL in full loop mode, and the temperature in the column was kept at 45 °C and that in the sample injector at 10 °C.

The different polyphenols were identified by comparison of their retention time, with those of the reference standards, the UV-Vis absorption spectra and the published data of the chromatographic behaviour of de compounds. Quantification analysis was performed by calibration curves of commercial standards using their specific wavelength at the UV-Vis absorption maximum or the corresponding to their aglycon when no standards were available.[9,10]

RESULTS AND DISCUSSION

The methanolic method was used to estimate the extraction capacity and selectivity, of phenolic compounds, from the leaves of *P. lentiscus*, *P. latifolia*, *G. alypum*, *C. siliqua*. The results showed a great variability in raw dry extracts of these four species. The extraction method can influence the extraction yield. Indeed, Erdogan-Orhan *et al.*, [11] carried out a Soxhlet extraction, of M. vulgare, using acetone, they obtained a dry extract yield significantly higher than that obtained by Ghedadba *et al.*,[12] who used dichloromethane. Thus, Ghedadba *et al.* reported that the extraction yield and the composition of the extracts vary according to the nature of the solvent, the period and the place of the harvest and the drying time.

Yield in dry extracts and contents of total phenols and flavonoids of the leaves of the four species

The effect of environmental degradation of the studied flora can be shown by the yield of the methanolic dry extract, total phenols and flavonoid contents of leaves of the four species, determined in three categories of collection plots. The results of the yields of dry extracts of *P. lentiscus, P. latifolia, G. alypum, C. siliqua* were summarized in Table1. These yields varied significantly ($p < 0.05$), between the sample collection plots. Indeed, the plant extracts of the plots of site 1 represent the highest yields, while low ones has been recorded in the plots of site 3. In general, the contents of dry extracts varied according to the species and to the method of extraction.

Table 1

Yield in dry extracts and contents of total phenols and flavonoids of the leaves of the four species, determined in three sites (S1, S2, S3) of Djebel-Zaghouan (National Park)

Species	Yield (%)			Total phenols (mg GAE/g DW)			Total flavonoids (mg QE/g DW)		
	S1	S2	S3	S 1	S2	S3	S1	S2	S3
P. lentiscus	18±1.1^a	14.0±1.4^b	12.79±1.3^c	1.05±0.1^c	287±1^b	5.46±0.1^a	0.69±0.1^c	1.77±0.1^b	3.39±0.2^a
P. latifolia	22±2.2^a	19.12±1.1^b	10.79 ±2.6^c	1.49±0.1^c	2.85±0.1^b	4.72±0.1^a	1.84±0.1^c	3.14±0.1^b	8.31±0.1^a
G. alypum	14.76±3.0^a	11.02±1.2^b	10.23±1.2^c	2.04±0.1^c	3.78±0.1^b	7.92±0.1^a	1.04±0.1^c	2.26±0.1^b	3.70±0.1^a
C. siliqua	12.03±1.3^a	10.37±1.2^b	9.73±1.1^c	2.50±0.2^c	4.38±0.2^b	6.45±0.2^a	3.42±0.5^c	6.75±0.4^b	7.42±0.2^a

Values in the same row with same letters are not significantly different at the 5% level (n = 3). S 1: recovery rate >53%; S 2: 20% <recovery rate <53%; S 3: recovery rate <20%; QE: quercetinequivalent; GAE: gallic acid equivalent; DW: dry weight

The results of the colorimetric assays of total phenols, have been recorded in Table 1. From these results, we can show that the studied species present very significantly variable contents ($p < 0.01$) in phenolic compounds, according to the gradient of degradation of the plots of collection. This heterogeneity is more marked in the plants harvested in the plots of site 3, where the highest

levels are recorded in G. *alypum* (7.92 mg GAE/g DW), followed by C. *siliqua* (6.45 mg GAE /g DW), by P. *lentiscus* (5.46 mg GAE/g DW) and P. *latifolia* (4.72 mg GAE/g DW), respectively. For flavonoids, the results show a similar trend as well as total phenols with a highly significant difference ($p<0.01$), between the plant collection plots. Indeed, extracts from site 3 plots give the highest levels, with 3.39 mg QE/g DW for *P. lentiscus*, 8.31 mg QE/g DW for *P. latifolia*, 3.70 mg QE/g DW for *G. alypum*, 7.42 mg QE/g DW). These results suggest that environmental degradation favoured the accumulation of flavonoids in the leaves of these species. The increase in polyphenol content, observed in the plots of site 2 and site 3, could be attributed to the mechanism of adaptation and survival of plants in these environments, which are hostile to them. Our results showed that the total phenol content varies from one plot to another for, the same plant. The variations observed could, probably, be due to many factors, in particular, climatic and environmental factors (temperature, altitude, sunshine and precipitation), geographical area, drought and diseases,[13,14] period of sample collection and stage of growth and development of the plant.[15] In fact, increased biosynthesis and accumulation of phenolic compounds, frequently occurs in plant tissues in response to biotic and abiotic stresses. These compounds can prevent oxidative modification by neutralizing free radicals, scavenging oxygen or decomposing peroxides, through their antioxidant activities1.[16] Genetic factors and plant growing conditions can play an important role in the production of secondary metabolites.[17,18] Other technical factors, in particular the extraction and the quantification methods, can also influence the estimation of the content of total phenols.[19] The total levels of phenols and flavonoids are high in the organs of the palm when the living environment of this species is not adequate. Indeed, in such situation the plant must set up a defense mechanism to ensure its growth and development.[20] The excessive production of phenols, the activation of antioxidant enzymes, in particular peroxidase (POD), superoxide dismutase (SOD) and catalases (CAT), are among these defense mechanisms.[21] The difference in phenolic content (including flavonoids) described in the literature can be attributed to several factors, namely the extraction method and the quantification method. The variability of polyphenol contents, in the medicinal species studied is, probably, due to the phenolic composition of the extracts, to the biotic conditions, such as the species, the organ or the physiological stage and to the abiotic conditions (season, climate and temperature).[22–25] Flavonoids are plant secondary metabolites with significant antioxidant and chelating properties. Next to phenolic compounds, the presence of flavonoids can also influence antioxidant capacity.[26,27]

Flavonoids are low molecular weight compounds found in legumes, fruits, flowers and leaves of plants, with several biological activities.[28] The polar extracts of the leaves of *Quercus ilex* showed a significant richness in flavonoids, in particular quercetin, isorhamnetin-3-O-glucopyranoside and phenolic ellagic acid.[29, 30]

Identification and quantification of phenolic compounds in the leaves of the four species.

The chromatographic analysis by UPLC-PDA-MS-tQ made it possible to identify and quantify eighteen compounds in *P. lentiscus*, twelve for *P. latifolia* and *G. alypum* and seventeen for *C. Siliqua* (Fig. 1, Table 2). The contents and nature of the main compounds varied from one plant to another and according to the collection plots. The results of the phenolic compositions of *P. lentiscus* leaves' showed that the main compounds were: digalloylquinic acid (3512.2–6018.2 µg/kg), trigalloylquinic acid (1275.4–2307.5µg/kg), verbascoside (98.6–1530.3 µg/kg) and myricetin-rhamnoside (656–1469.4 µg/g). These results revealed that the leaves of *P.lentiscus*were relatively rich in phenolic acids (gallic, galloylquinic, digalloylquinic and trigalloylquinic), inphenols (verbascoside and isoverbascoside) andin flavonoids, more particularly flavonols (quercetin, kaempferol), flavan-3-ol (catechin) and flavones (apigenin, luteolin) (Table 2). On the other hand, the results showed that *P. latifoli* leaves' were characterized by the presence of these major compounds: verbascoside (1085.6–2766.3 µg/kg), luteolin-hexose (2072.8–2522.1 µg/kg), apigenin-diglycoside (723.2–995.4 µg/kg) and quercetin-3-*O*-rutinoside (607.2–757.1 µg/kg) (Table 2). Verbascoside accumulation (2766.3 ± 860.4 µg/kg) is more pronounced in site 3 plots than in site 2 and 1 plots. The results showed high concentrations ofapigenin-diglycoside (995.4 ± 38.3 µg/kg), verbascoside derivative (444.4 ± 15.9 µg/kg), digalloylglucose (264.0 ± 6.9 µg/kg), gentisic acid glycoside (221.5 ± 10.2 µg/g), quercetin-hexose (168.2 ± 8.1 µg/kg) and digalloylquinic acid

(72.0 ± 3.5 µg/kg) (Table 2). Thus, plots from site 1 showed higher concentrations of flavonoids, belonging to different structures, such as flavones, represented by luteolin-hexose (2522.1 ± 247.3 µg/kg) and luteolin-diglycoside (230.6 ± 19.9 µg/kg) and the flavonols, represented by quercetin-3-O-rutinoside (757.1 ± 72.8 µg/kg). On the other hand,we have found that the major compounds in *G. alypum* were verbascoside (1388.1–2995.7 µg/kg), luteolin-hexose (1158.4–1286.2 µg/kg), apigenin-diglycoside (788.9–932.3 µg/kg), verbascoside derivative (88.4–857.2 µg/kg), quercetin-3-*O*-rutinoside (254.9–436.7 µg/kg) and digalloylglucose (247.2–317.8 µg/kg) (Table 2).The contents of the phenolic compounds in*G. alypum* depend significantly of the collection plots. In stage 3 plots, verbascoside (2995.7 ± 210.9 µg/kg), luteolin-hexose (1286.2 ± 90.6 µg/kg), quercetin-3-*O*-rutinoside (436.7 ± 28.0 µg/kg) and digalloylglucose (317.8 ± 1.9µg/g), were the main compounds identified in the extracts. Extracts from site 2 plots were characterized by apigenin-diglycoside (932.3 ± 117.2 µg/kg), luteolin-diglycoside (229.7 ± 0.7 µg/kg), isoverbascoside (94.8 ± 13.1 µg/kg), kaempherol-rutinoside (75.2 ± 12.0 µg/kg) and digalloylquinic acid (64.4 ± 2.4 µg/kg). In site 1 plots, the verbascoside derivative (857.2 ± 6.3 µg/kg) was predominant (Table 2). In addition, tetragalloylglucose (4144.4–6126.5 µg/kg), myricetin-rhamnoside (3983.8-5242.6 µg/kg), digalloylglucose (1498.5–1941.7 µg/kg), pentagalloylglucose (843–1232 µg/kg), quercetin-3-*O*-rhamnoside (677.5–888.1 µg/kg) and myricetin-hexose (565.9–753 µg/kg), were the major compounds. *C. siliqua* leaves, in stage 3, plots are richer in tetragalloylglucose (6126.5 ± 575.2 µg/kg), myricetin-rhamnoside (5242.6 ± 76.4 µg/kg), digalloylglucose (1941.7 ± 153.0 µg/g) and pentagalloylglucose (1232.0 ± 102.1 µg/kg). These results revealed that *C. siliqua* leaves extract contain both phenolic acids (gallic acid) and flavonoids (Table 2). The analysis of variance showed a significant effect ($p < 0.05$) of site degradation, on the levels of phenolic compounds.

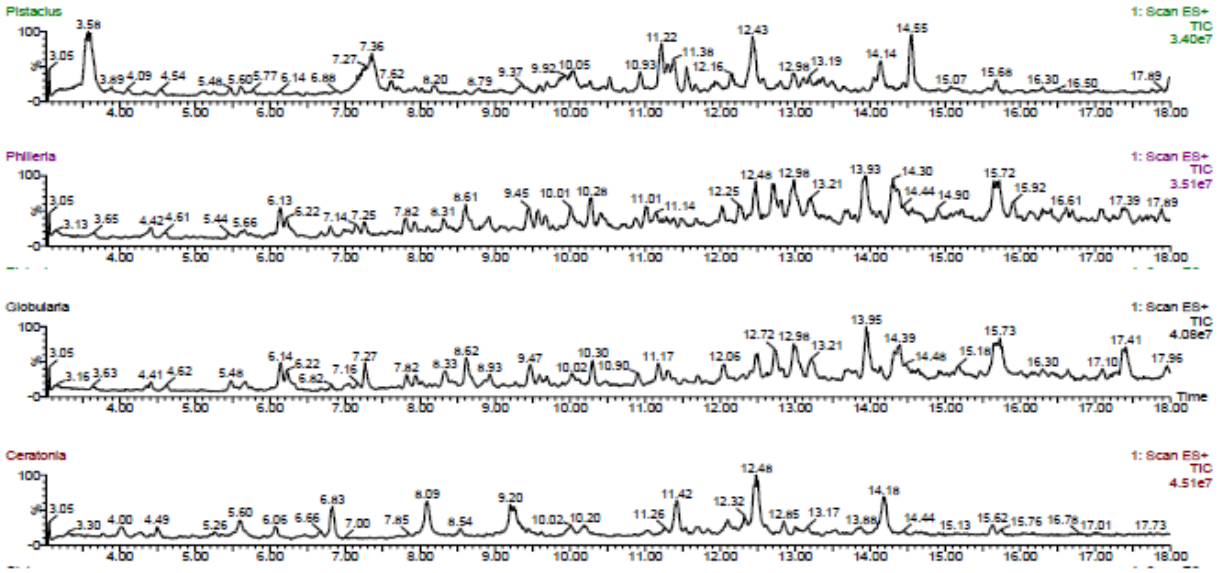

Fig. 1– Example of UPLC-PDA-MS-tQ chromatogram in TIC mode of the four plant species.

Mean composition of the phenolic compounds contained in the studied species based on sample collection plots

The comparison between the studied species is presented in Table 3. The results showed variability between them, in terms of nature and quantity of phenolic compounds, with the presence of a single compound in common (Luteolin- hexose), chemical compound of the flavonoid family and more specifically one of the most common flavones. Luteolin is a flavonoid that is loaded with antioxidant properties. It is present in food items like vegetables, leafy greens, citrus fruits, and spices. Luteolin can boost cardiovascular health, protect the brain and nerves, reduce inflammation

and allergies, and promote testosterone levels. *P. latifolia* and *G. alypum* are similar in phenolic composition. *P. lentiscus* and *C. siliqua* have eight compounds in common (Table 3).

Our results also showed that the extracts, obtained, present different significant concentrations of phenolic compounds, which vary according to the sampling plots of the species and the climatic and geographical conditions. This is in agreement with several studies, which have shown that climatic conditions (altitudes, climate) play a decisive role in the extraction capacity of phenolic compounds,[31,32] Scalbert *et al.*[33] showed that phenolic compounds contain 9 groups of phenols: stibenes, lignans, phenolic acids, flavonols, isoflavones, flavonones, anthocyanidins, ellagitannis and proanthocyanidins, which are characterized by their very varied chemical structures.

C. siliqua has been shown to present a potential reservoir of bioactive natural molecules. This species also contains phenolic compounds, which give it different antioxidant roles.[20,34,35] Rodríguez-Pérez *et al.*[36] showed that most of the compounds, identified in the leaves of *Pistacia lentiscus*, were flavonoids, phenolic acids and their derivatives. Myricetin glycoside, catechin β-glucogallin and quercitrin gallate, are the major compounds of this species. The results of the work of Sacchetti *et al.*[37] and Fadili *et al.*[38] established that rosemary (*Rosmarinus Officinalis* L.), exhibits significant antioxidant properties, attributed to the phenolic compounds and flavonoids present in this plant. The molecular characterization of the leaves of P. *angustifolia* showed the presence of active biological compounds, in particular phenols and flavonoids.

Statistical analysis

All analysis were repeated three times, for each sample, and expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA), followed by Duncan's multiple range test, was applied to compare the means, at the significance level $p < 0.05$. All analysis was performed using the SAS v 9.1 software package.

CONCLUSION

The results of this study showed that the total phenol and flavonoids contents of the studied samples varied from one plant to another and according to the sites of collection. There is a wide variability of polyphenolic content, not only by species, but also depending on the polyphenols type. Differences, between the four studied species, in composition and quantity of phenolic compounds in the leaves, were revealed. Polyphenols are important natural antioxidants, which play a major role in the prevention of various pathologies. All the results of the present study should be used for subsequent investigations on the pharmacological potentials of the plant species studied. Priority should be given to the isolation and purification of active compounds from these plants.

Acknowledgments. We thank the Ministry of Higher Education and Scientific Research, Tunisia as well as Ministry of Agriculture, for their financial are grateful and Professor Mohamed Rigane for useful discussions about the English.

REFERENCES

1. S. M. Memarzadeh, A. Gholami, A. G. Pirbalouti and S. Masoum, *Ind. Crops Prod.*, **2020,** *154*, 112719.
2. C. G. Awuchi, V. S. Igwe and I. O. Amagwula, *J. Adv. Acad. Res*., **2020**, *6*, 1.
3. C. Jain, S. Khatana and R. Vijayvergia, *Int. J. Pharm. Sci. Res*., **2019**, *10*, 494.
4. H. Nawaz, T. Mushtaq and M. Yaqoob, *Int. J. Poult. Sci*., **2006**, *43*, 388.
5. B. L. Queen and T. O. Tollefsbol, *Curr. Aging. Sci*., **2010**, *3*, 34.
6. P. Ljubuncic, I. Portnaya, U. Cogan, H. Azaizeh and A. Bomzon, *J. Ethnopharmacol.*, **2005**, *101*, 153.
7. A. Delpino-Rius, J. Eras, F. Vilaró, M.A. Cubero, M, Balcells and R. Canela-Garayoa, *Food Chem.*, **2015**, *172*, 574
8. A. Delpino-Rius, J. Eras, F. Gatius, M. Balcells and R. Canela-Garayoa, *Molecules*, **2019**, *24*, 3289.
9. S. Fkiri, B. Essghaier, B. Stiti, H. Achour, L. Ben Slimane, R. Ben Salem, Z. Nasr, R. Ksouri, G. Rigane, and A. Khaldi, *Chem. Biodivers.*, **2023**.
10. A. Mahdhi, H. Ghazghazi, M. El Aloui, R. Ben Salem, and G. Rigane, *Nutr. Food Sci*., **2021**, *9*, 1907.
11. E. Erdogan-Orhan, R. Belhattab, F. S. Senol, A. R. Gülpinar, S. Hosbas and M. Kartal, *Ind. Crop. Prod*., **2010**, *32*, 566.
12. N. Ghedadba, L. Hambaba, M. C. Aberkane, S. M. Oueld-Mokhtar and H. Nassima Fercha, *J. Nat. Prod.* **2014,** *2*, 64.
13. N. S. Ebrahimi, J. Hadian, M. H. Mirjalili, A. Sonboli and M. Yousefzadi, *Food Chem.*, **2008**, *110*, 927.
14. N. Andarwulan, R. Batari, D. A. Sandrosari, B. Bolling and H. Wijaya, *Food Chem.*, **2010**, *121*, 1231.
15. G. Miliauskas, P. R. Venskutonis and T. A. Van Beek, *Food Chem.*, **2004**, *85*, 23.
16. R. J. Nijveldt, E. Nood, D. E. Hoorn, P. G. Boelens, K. Norren and P. Leeuwen, *Am. J. Clin. Nutr*., **2001**, *74*, 418.
17. M. S. Islam, M. Yoshimoto, K. Ishigure, S. Okuno and O. Yamakawa, *J. Am. Soc. Hortic. Sci*., **2003,** *128*, 182.
18. A. Hashempour, R. F. Ghazvini, D. Bakhshi and S. A. Sanam, *Aust. J. Crop Sci.*, **2010**, *4*, 258.

19. K. W. Lee, Y. J. Kim H. J. Lee and C. Y. Lee, *Food Chem*., **2003**, *51*, 7292.
20. F. Fadel, S. Fattouch, S. Tahrouch, R. Lahmar, A. Benddou and A. Hatimi, *J. Mater. Environ. Sci.*, **2011**, *2*, 285.
21. F. Morina, L. Jovanovic, M. Mojovic, M. Vidovic, D. Pankovic and S. Veljovic Jovanovic, *Physiol. Plant*, **2010**, *140*, 209.
22. M. Luczkiewicz, W. Cisowski, P. Kaiser, R. Ochocki and A. Piotrowski, *Acta Pol. Pharm.*, **2001**, *58*, 373–379.
23. S. Vicas, D. Rugina and C. Sosaciu, *Agriculture Cluj-Napoca*, **2008**, *65*, 327.
24. M. Locatelli, F. Travaglia, J. D. Coisson, A. Martelli, C. Stevigny and M. Arlorio, *Food Chem.*, **2010**, *119*, 1647.
25. L. Amjad and M. Shafighi, *Int. J. Agric. Crop Sci*., **2013,** *5*, 1133.
26. F. Sharififar, G. Dehghn-Nudeh and M. Mirtajaldini, *Food Chem.*, **2009**, *112*, 885.
27. M. S. Stanković, *Kragujevac J. Sci.*, **2011**, *33*, 63.
28. J. B. Harborne and C. A. Williams, *Phytochem*., **2000**, *55*, 481.
29. A. Karioti, A. R. Bilia, C. Gabbiani, L. Messori and H. Skaltsa, *Tetrahedron Lett.*, **2009**, *50*, 1771.
30. A. Karioti, A. R. Bilia and H. Skaltsa, *Food Chem.*, **2010**, *123*, 131.
31. H. Toberman, C. D. Evans, C. Freeman, N. Fenner, M. Whit, B. A. Emmet and R. R. E. Artz, *Soil Biol. Biochem.*, **2008**, *40*, 1519.
32. K. Msaâda K. Hosni, M. BenTaarit, M. Hammami and B. Marzouk, *Sci. Hortic.*, **2009**, *120*, 525.
33. A. Scalbert and G. Williamson, *J. Nutr.*, **2000**, *130*, 2073S.
34. H. E. Hajaji, N. Lachkar, K. Alaoui, Y. Cherrah, A. Farah, A. Ennabili, B. E. Bali and M. Lachkar, *Arab. J. Chem.*, **2011**, *4*, 321.
35. A. B. Hsouna, M. Saoudi, M. Trigui, K. Jamoussi, T. Boudawara, S. Jaoua and A. E. Feki, *Food Chem. Toxicol*., **2011**, *49*, 3183.
36. C. Rodríguez-Pérez, R. Quirantes-Piné, N. Amessis-Ouchemoukh, K. Madani, A. Segura-Carretero and A. A. Fernández-Gutierrez, *J. Pharm. Biomed. Anal.*, **2013**, *77*, 167.
37. G. Sacchetti, S. Maietti, M. Muzzoli, M. Scaglianti, S. Manfredini, M. Radice M and R. Bruni, *Food Chem.*, **2005**, *91*, 621.
38. K. Fadili, S. Amalich, S. K. N'dedianhoua, M. Bouachrine, M. Mahjoubi, F. El Hilali and T. Zair, *Int. J. Innov. Sci. Res.*, **2015**, *17*, 24.

Table 2

Content (µg/kg DW) and phenolic compounds, identified in the extract of the leaves of *P. lentiscus*, *P.latifolia*, *G. alypum* and *C. siliqua*, in three categories of collection plots

Rt	Compounds	*Pistacialentiscus*			*Phillyrealatifolia*			*Globulariaalypum*			*Ceratoniasiliqua*		
		S1	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**	**SsSS1**	**S2**	**S3**
2.92	Gallic acid	214.3±9.3^{c}	281.9±10.2^{b}	403.6±12.2^{a}	ND	ND	ND	ND	ND		344.8±7.3^{c}	396.1±12.4^{b}	425.7±15.1a
4.60	Galloylquinic acid	459.7±13.4c	696±14.5b	1082.4±70.3a	51.6±4.4c	72±3.5a	59.5±3.1b	57.7±4.6c	64.4±2.4a	62.4±4.1b	ND	ND	ND
5.63	Digalloylglucose	ND	ND	ND	136.2±12.3c	264±6.9a	175.2±17.4b	247.2±8.6c	303.3±10.4b	317.8±1.9a	1498.5±14.2c	1712.6±96.5b	1941.7±153a
6.16	Gentisic acid glycoside	ND	ND	ND	74.1±10.6b	221.5±10.2a	67.1±7.2c	37.4±3.1c	68.9±9.1b	83.6±3.3a	ND	ND	ND
7.39	Digalloylquinic acid	3512.2±410.1c	4792.6±434.5b	6018.2±205.7a	ND	ND	ND	ND	ND	ND	ND	ND	ND
8.23	Catechin	154.2±7.1a	121.8±6.4c	146±7.2b	ND	ND	ND	ND	ND	ND	ND	ND	ND
9.98	Trigalloylquinic acid	1275.4±90.4b	893.287.2c	2307.5±140.4a	ND	ND	ND	ND	ND	ND	ND	ND	ND
11.25	Myricetin-diglycoside	279.9c±10.6	539±14.1b	870.4±71.2a	ND	ND	ND	ND	ND	ND	47±3.7c	52.9±5.2b	57.5±12..3a
11.34	Myricetin-glucuronide	71.3±7.8c	125.8±10.6b	239.5±11.6a	ND	ND	ND	ND	ND	ND	ND	ND	ND
11.34	DerivativeVerbascoside	ND	ND	ND	74±7.1c	444.4±15.9a	75.4±6.3b	857.2±6.3a	741.4±14.3b	88.4±6.5c	ND	ND	ND
11.42	Myricetin-hexose	98.2±1O.11c	177±11.3b	311.1±12.3a	ND	ND	ND	ND	ND	ND	565.9±12.4c	667.6±22.3b	753±19.4a
12.09	Tetragalloylglucose	ND	ND	ND	ND	ND	ND	ND	ND	ND	4144.4±121.4c	4932±88.7b	6126.5±575.2a
12.19	Myricetin-arabinoside	63.2±7.3c	107.4±9.4b	136.1±8.2a	ND	ND	ND	ND	ND	ND	124.5±8.3c	146.9±17.9b	165.3±10.3a
12.33	Myricetin-rhamnoside	656.0±151.2c	985.3±210.6b	1469.4±140.5a	ND	ND	ND	ND	ND	ND	3983.8±144.6	4601±102.6	5242.6±76.4a
12.52	Quercetin-3-O-rutinoside	ND	ND	ND	757.1±72.8a	723.6±21.6b	607.2±13.5c	254.9±7.4c	427.6±12.5b	436.7±28.0a	ND	ND	ND
12.61	Quercetin-3-O-galactoside	69.2±6.9c	107.2±12.5a	78.3±10.7b	ND	ND	ND	ND	ND	ND	60.5±3.4c	67.7±12.7b	74.8±6.2a

Rt	Compounds	*Pistacialentiscus*			*Phillyrealatifolia*			*Globulariaalypum*			*Ceratoniasiliqua*		
		S1	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**	**SsSS1**	**S2**	**S3**
12.75	Luteolin-diglycoside	ND	ND	ND	230,6±19.9a	225,7±11.4b	188,8±14.2c	181,7±12.3c	229,7±0.7a	202,9±10.1b	ND	ND	ND
12.80	Quercetin-3-O-glucoside	118.4±9.3a	73.4±8.9c	95.6±13.6b	ND	ND	ND	ND	ND	ND	112±10.6c	127.4±14.3b	142.7±8.4a
12.85	Quercetin-hexose	ND	ND	ND	150.5±13.3b	168.2±8.1a	131.3±8.4c	89.9±3.1b	47.8±2.2c	121.7±8.3a	ND	ND	ND
13.02	Luteolin-hexose	208.6±9.6a	135.3±12.9b	131.5±14.9c	2522.1±247.3a	2400.5±22.8b	2072.8±151.6c	1158.4±22.7c	1199±43.7b	1286.2±90.6a	13.6±11.4a	13.2±2.7a	13.8±2.4a
13.23	Verbascoside	1530.3±99.6a	98.6±8.1c	178.1±20.6b	1085.6±101.3c	2095.6±18.3b	2766.3±860.4a	1388.1±21.8c	1760.6±47.3b	2995.7±210.9a	ND	ND	ND
13.36	Pentagalloylglucopyranose	ND	ND	ND	ND	ND	ND	ND	ND	ND	373.6±27.3c	453.6±8.1b	537.6a
13.40	Quercetin-O-glucoside	47.4±c6;8	77.3±7.5b	96.6±13.3a	ND	ND	ND	ND	ND	ND	ND	ND	ND
13.69	Kaemphérol-rutinoside	185.2±11.7a	26.3±15.3b	24.9±12.8c	67.3±6.4a	75.3±3.6b	55.7±6.8c	51.1±5.6c	75.2±12.0a	74.4±5.3b	ND	ND	ND
13.86	Quercetin-glucuronide	ND	ND	ND	ND	ND	ND	ND	ND	ND	105.6±4.9c	124.4±11.3b	141.5a
13.90	Pentagalloylglucose	ND	ND	ND	ND	ND	ND	ND	ND	ND	843±9.1c	1092.3±119.5b	1232±102.1a
14.06	Kaemphérol-hexose	ND	ND	ND	ND	ND	ND	ND	ND	ND	22±3.1c	23.9±3.4b	26.2a
14.17	Isoverbascoside	ND	ND	ND	78.1±4.3b	189.0±6.2a	71.7±5.7c	64.6±2.6c	94.8±13.1a	76.2±2.9b	ND	ND	ND
14.18	Quercetin-3O-rhamnoside	ND	ND	ND	ND	ND	ND	ND	ND	ND	677.5±21c	780±13.7b	888.1a
14.40	Apigenin-diglycoside	259.8±12.9c	407.5±10.7b	562.5±78.2a	984.4±21.4b	995.4±38.3a	723.2±12.4c	788.9±11.9b	932.3±117.2a	789.4±13.2b	ND	ND	ND
15.62	Kaemphérol-deoxyhexose	ND	ND	ND	ND	ND	ND	ND	ND	ND	69.4±3.1c	78.5±5.9b	88.5a
17.39	Derivative Luteolin	48.9±7.1c	51.8±8.4b	58.9±8.2a	ND	ND	ND	ND	ND	ND	19.2±3.4b	19.9±3.8b	22.7±1.7a

Values in the same row with the same letters are not significantly different at the 5% level (n = 3). Stage 1: recovery rate > 53%; Stage 2: 20% <recovery rate < 53%; Stage 3: recovery rate < 20%; DW: dry Wieght. ND: Not Detected, Rt: Retention time expressed in min.

Table 3

Mean composition of the phenolic compounds contained in the extracts of the leaves of *P. lentiscus*, *P. latifolia*, *G. alypum* and *C. siliqua*,based on sample collection plots

Rt	Phenolic Compounds	*P. lentiscus*	*P. latifolia*	*G. alypum*	*C. siliqua*
		Mean(µg/kg)	**Mean**(µg/kg)	**Mean**(µg/kg)	**Mean**(µg/kg)
2.92	Gallic acid	299.93±95.93	ND	ND	388.87±40.93
4.60	Galloylquinic acid	746.03±314.35	60.2±8.56	61.5±3.44	ND
5.63	Digalloylglucose	ND	205.65±60.23	289.43±37.29	1717.60±221.64
6.16	Gentisic acid glycoside	ND	100.025±82.53	63.30±23.60	ND
7.39	Digalloylquinic acid	4774.33±1253.10	ND	ND	ND
8.23	Catechin	140.67±16.85	ND	ND	ND
9.98	Trigalloylquinic acid	1492.03±731.61	ND	ND	ND
11.25	Myricetin-diglycoside	563.10±295.99	ND	ND	52.47±5.26
11.34	Myricetin-glucuronide	145.53±85.82	ND	ND	-
11.34	DerivativeVerbascoside	ND	362.75±372.87	562.33±414.50	ND
11.42	Myricetin-hexose	195.43±107.64	ND	ND	662.17±93.67
12.09	Tetragalloylglucose	ND	ND	ND	5067.63±997.99
12.19	Myricetin-arabinoside	102.23±36.72	ND	ND	145.57±20.43
12.33	Myricetin-rhamnoside	1036.90±409.15	ND	ND	4609.13±629.44
12.52	Quercetin-3-O-rutinoside	ND	585.7±229.70	373.07±102.44	ND
12.61	Quercetin-3-O-galactoside	84.90±19.84	ND	ND	67.67±7.15
12.75	Luteolin-diglycoside	ND	206.7±25.02	204.77±24.05	ND
12.80	Quercetin-3-O-glucoside	95.80±22.50	ND	ND	127.37±15.35
12.85	Quercetin-hexose	ND	134.975±33.62	86.47±37.07	ND
13.02	Luteolin-hexose	158.47±43.46	2038.45±616.62	1214.53±65.30	13.53±0.31
13.23	Verbascoside	602.33±804.63	1833.9±752.01	2048.13±841.49	ND
13.36	Pentagalloylglucopyranose	ND	ND	ND	454.93±82.01
13.40	Quercetin-O-glucoside	73.77±24.79	ND	ND	ND
13.69	Kaemphérol-rutinoside	78.80±92.15	62.35±11.00	66.90±13.69	ND
13.86	Quercetin-glucuronide	ND	ND	ND	123.83±17.96
13.90	Pentagalloylglucose	ND	ND	ND	1055.77±197.06
14.06	Kaemphérol-hexose	ND	ND	ND	24.03±2.10
14.17	Isoverbascoside	ND	100.85±59.02	78.53±15.23	ND
14.18	Quercetin-3-O-rhamnoside	ND	ND	ND	781.93±105.31
14.40	Apigenin-diglycoside	409.93±151.36	872.975±137.73	836.87±82.65	ND
15.62	Kaemphérol-deoxyhexose	ND	ND	ND	78.80±9.55
17.39	DerivativeLuteolin	53.20±5.14	ND	ND	20.60±1.85

ND: Not Detected, Rt: Retention time expressed in min.