doi.org/10.1002/cbdv.202200237

RESEARCH ARTICLE

CHEMISTRY & BIODIVERSITY

Special Collection

Ziziphus lotus (L.) Lam. as a Source of Health Promoting Products: Metabolomic Profile, Antioxidant and Tyrosinase Inhibitory Activities

Anna Cacciola,[a, b] Valeria D'Angelo,[a] Francesco M. Raimondo,[c] Maria Paola Germanò,*[a] Alessandra Braca,[d, e] and Marinella De Leo[d, e]

[a] Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali – ChiBioFarAm, Viale Ferdinando Stagno d'Alcontres, 31 vill. S. Agata, 98166 Messina, Italy, e-mail: germanom@unime.it

[b] Fondazione 'Prof. Antonino Imbesi', Piazza Pugliatti 1, 98122 Messina, Italy

[c] PLANTA/Centro autonomo di Ricerca, Documentazione e Formazione, Via Serraglio Vecchio 28, 90123 Palermo, Italy

[d] Dipartimento di Farmacia, Università di Pisa, via Bonanno 33, 56126 Pisa, Italy

[e] CISUP, Center for Instrumentation Sharing, Pisa University,Lungarno Pacinotti 43/44, 56126 Pisa, Italy

The methanolic extract of the stem bark of a wild species of jujube, *Ziziphus lotus* (L.) Lam., growing in Sicily, was chemically and biologically investigated. The chemical profile was defined by UHPLC-HR-ESI-Orbitrap/MS analysis whereas antioxidant and tyrosinase inhibitory activities were investigated by *in vitro* assays. The extract showed a high total phenolic and flavonoid content (TPC = 271.65 GAE/g and TFC = 188.11 RE/g extract). Metabolomic analysis revealed a rich phytocomplex characterized by phenols, cyclopeptide alkaloids, and triterpenoid saponins, some of which here detected for the first time. The mushroom tyrosinase inhibition assay displayed that the methanolic extract efficiently inhibits the monophenolase and diphenolase activity. Furthermore, the extract showed a strong ability to scavenge DPPH, a good Fe^{3+} reducing antioxidant power, in addition to a Fe^{2+} chelating activity. Taken together, these results suggest possible novel applications of wild jujube stem bark as a source of potential skin-care agents with several uses in pharmaceutical and cosmetic industries.

Keywords: wild jujube (*Ziziphus lotus*) stem bark, polyphenols, saponins, UHPLC/MS, tyrosinase inhibitory activity.

Introduction

Ziziphus genus (Rhamnaceae) includes many species that grow in arid and semiarid areas widely distributed throughout the Mediterranean basin.

Different *Ziziphus* spp. have been used for thousands of years in traditional medicine for the treatment of urinary troubles, diabetes, skin infections, fever, diarrhea, insomnia, bronchitis, and as sedative and hypoglycemic agents.[1,2] Many studies have also reported the presence of various chemical constituents belonging to different classes of secondary metabolites e.g., polyphenols, alkaloids, terpenoids, saponins.[1]

Among different species, *Z. lotus* (L.) Lam. has shown considerable attention for its phytochemical and pharmacological properties. Previous chemical studies on Tunisian and Jordan plants afforded to the isolation of dammarane saponins and cyclopeptide alkaloids from the root bark[3–6] and leaves,[7,8] while phenolic compounds have been recently detected by LC/MS technique in different parts (branches, leaves, roots, and stem bark) of an Algerian sample.[9] Moreover, pharmacological investigations on *Z. lotus* crude extracts and isolated compounds showed a wide range of *in vitro* and *in vivo* effects, including antimicrobial, antidiarrheal, anti-inflammatory, analgesic, antioxidant, and hepatoprotective activities.[1,9] In addition, potential dermatoprotective properties of

extracts from the leaves and fruits have been also reported due to their strong tyrosinase inhibitory activity.[10] Tyrosinase (TYR) is the key enzyme that catalyzes the first two steps in mammalian melanogenesis. It is a glycosylated, and copper-containing oxidase, which catalyze the oxidations of monophenols and *o*-diphenols into reactive intermediate product *o*-quinone (dopaquinone), which is further oxidized into eumelanin and pheomelanin through other enzymatic and non-enzymatic reactions.[11] Melanin is responsible for absorbing light to protect skin cells from UVB-radiation damage. Since tyrosinase-catalyzed reactions are highly associated with hyperpigmentation, TYR inhibition is one strategy aimed at controlling the production of melanin. Therefore, TYR inhibitors (TYRIs) have become important dermatoprotective targets. Several well-known TYRIs as hydroquinone, arbutin, kojic acid, ascorbic acid, and other molecules from natural sources are anti-melanin and whitening agents. Nowadays, the discover of new TYR inhibitors (TYRIs) has gained high interest in the therapies of skin pathologies as well as in dermocosmetic treatments. In this context, the efficacy of new anti-melanogenic agents from plant sources was evaluated in our previous research works.[12,13]

Considering that the most common targets of investigations on *Ziziphus* spp. are the leaves, fruits, and roots, in this study we focused the attention to the stem bark of an endemic species of *Z. lotus* growing in Sicily. To the best of our knowledge, the potential skin whitening properties of *Z. lotus* stem bark extract is now investigated for the first time. In addition, as the oxidative stress induces an abnormal ROS formation leading to skin photoaging, the antioxidant and free radical scavenging activities were also evaluated by *in vitro* cell free colorimetric assays. Moreover, an in-depth metabolomic study was performed to identify the bioactive compounds, which as antioxidants and tyrosinase inhibitors, may be of interest for both pharmaceutical and food industry.

Results and Discussion

Total Phenolic and Flavonoid Content

Total phenols (TPC) and flavonoids (TFC) were measured in the methanolic extract of wild jujube stem bark. Results were reported in *Table 1*. TPC determined with the Folin-Ciocalteu reagent was 271.65 ± 5.60 mg gallic acid equivalents (GAE)/g of extract. TFC detected by aluminum chloride method was 188.11 ± 7.48 mg rutin equivalents (RE)/g of extract. All parts of *Z. lotus* resulted rich in polyphenols and flavonoids. However, several factors may affect the content of phenolic compounds, such as phenological stage, climatic conditions, methods of extraction, geographical location.[9] It is widely known that leaves and fruits have the highest phenolic content. On the other hand, the woody vascular parts, especially barks, may be considered an important source of polyphenolic compounds with potential biological effects.[14] Accordingly, stem bark samples of *Z. lotus* from Algeria revealed a high total amount of polyphenols (750.38 mg GAE/g extract).[15] Meanwhile, previous investigations on other species, *Z. mauritiana* Lam. and *Z. mucronate* Willd., reported a low content (< 100 mg/g of extract).[16,17]

Antioxidant Activity

High antioxidant potential is most frequently related to high phenolic content and bioavailability of the most bioactive compounds.[18] For this reason, *in vitro* assays to assess the antioxidant potential of wild jujube were performed. To have a realistic evaluation of the antioxidant ability, three assays based on different mechanism of reaction, DPPH, FRAP, and ferrous ion chelating power were carried out. Results, summarized in *Table 1*, showed a high DPPH scavenging ability (304.02 ± 4.80 mg ascorbic acid equivalents, AAE/g extract) and a strong reducing power in the FRAP assay (296.68 ± 1.81 mg Trolox equivalents, TE/g extract). Moreover, by measuring metal chelating activity, the extract demonstrated a strong capacity to

Table 1. Total phenolic and flavonoid content, DPPH free radical scavenging and metal chelating activity of *Z. lotus* stem bark methanolic extract.

	TPC (mg GAE/g)	TFC (mg RE/g)	DPPH (mg AAE/g)	Metal chelating (mg EDTAE/g)	FRAP (mg TE/g)
Methanolic Extract	271.65 ± 5.60	188.11 ± 7.48	304.02 ± 4.80	39.01 ± 4.30	296.68 ± 1.81

Results are reported as mean ± SD ($n = 3$), GAE = Gallic acid equivalents, RE = Rutin equivalents, AAE = Ascorbic acid equivalents, EDTAE = Ethylenediaminetetraacetic acid equivalents, TE = Trolox equivalents.

chelate ferrous ions from ferrozine complex (39.01 ± 4.30 mg ethylenediaminetetraacetic acid equivalents, EDTA/g extract). In order to in deep analyze the parameters evaluated, a Pearson correlation was carried out. Positive correlation coefficients (≥ 0.935) were found between TPC, TFC, and all antioxidant assays although without any statistically significant differences.

The antioxidant effects of *Ziziphus* spp. stem bark extracts have also been evaluated through various assays in previous studies. Thus, for *Z. mucronata*, ABTS, DPPH, and ferrous reducing antioxidant property (FRAP) methods were performed. Results indicated a correlation between the antioxidant activity and the total phenolic content.[16] In an earlier report, the antioxidant capacity of *Z. mauritiana* stem bark resulted higher than the seed extract by the phosphomolybdenum method.[17] Similarly, the free radical scavenging potential of *Z. nummularia* stem bark was also evaluated in the DPPH assay for developing new and safe natural antioxidant extract.[19] According to our results, the methanolic extract of *Z. lotus* samples harvested in Algeria exhibited a strong free radical scavenging activity expressed by equivalent of ascorbic acid (480.20 mg/g extract), confirming that the stem bark may be considered a good source of antioxidant compounds available throughout the year.[15]

Conversely, no data are available in the literature on metal chelating ability of *Ziziphus* spp. stem bark. Therefore, in the present study the investigated extract exhibited a strong capacity to chelate ferrous ions providing an additional value to the antioxidant activity.

Tyrosinase Inhibition

Melanin production in the living organism is a natural adaption to the outside environment for protecting the skin from ultraviolet damage and reactive oxygen species (ROS).

Tyrosinase is the type-3 metalloenzyme, which play a key role in melanin production. Therefore, TYRs are used in cosmetic and medicinal industries to prevent or treat overproduction of melanin such as melasma, solar lentigo, and post inflammatory melanoderma, and could be used in food industry to prevent undesired browning of fruits and vegetables.[20]

Most of the strategies of controlling melanin production have focused on the regulation of TYR activity. Tyrosinase catalyzes two steps with monophenolase and diphenolase at the same active site: the hydroxylation of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) and the oxidation of L-DOPA to dopaquinone; thus, the inhibitory effect on melanogenesis could be estimated on both monophenolase and diphenolase activity.

Our results showed that the wild jujube methanolic extract efficiently and dose-dependently inhibited both monophenolase and diphenolase (*Figure 1*). To compare the obtained results the IC_{50} values were calculated and summarized in *Table 2*. Kojic acid, a known whitening agent, was used as positive control. According to a previous study on fruits and leaves,

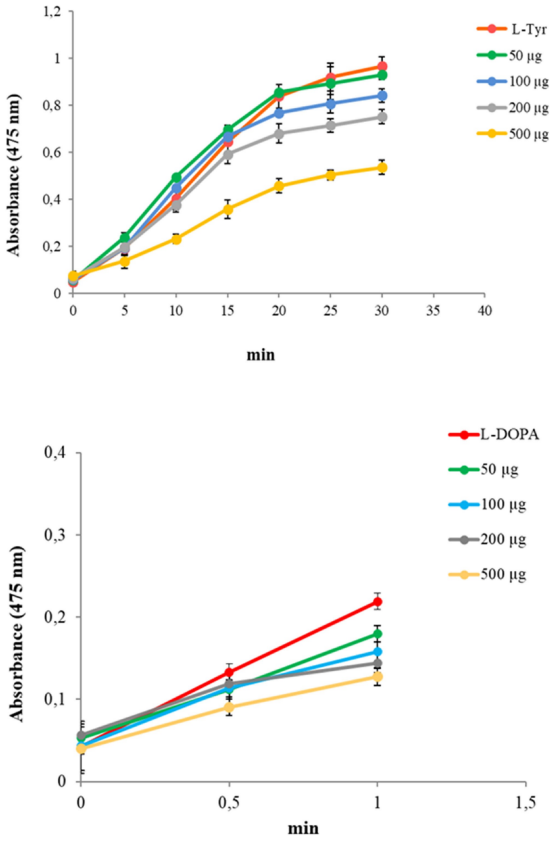

Figure 1. Kinetic curves of *Z. lotus* stem bark methanolic extract (50–500 µg/mL) on TYR monophenolase (A) and diphenolase (B) activity. Results are reported as mean ± SD ($n = 3$).

Table 2. Inhibitory effects on tyrosinase (IC_{50}) of *Z. lotus* stem bark methanolic extract.

Sample	Monophenolase (IC_{50} µg/mL)	Diphenolase (IC_{50} µg/mL)
Methanolic extract	333.15 ± 10.34	110.35 ± 12.90
Kojic acid	32.68 ± 1.50	2.24 ± 0.18

Results are reported as mean ± SD ($n = 3$).

Z. lotus represented a rich source of antimelanogenic agents, due to its high polyphenolic content,[10] whereas our study reported for the first time that even the stem bark could be of interest for the isolation of whitening compounds. In addition, as it is known that copper ions are present in the TYR pocket, the good metal chelating ability of the investigated extract, may be involved in the enzyme inhibition mechanism.

Metabolomic Analysis of Stem Bark Methanolic Extract

The chemical characterization of the methanolic extract of *Z. lotus* stem bark was performed by ultra-high-performance liquid chromatography (UHPLC) coupled to a high resolution-mass spectrometer (HR-MS) equipped with an electrospray ionization source (ESI) in both positive and negative ionization modes, as illustrated by chromatograms in *Figure 2*. The extract showed a very complex chemical profile characterized by the presence of three main classes of metabolites, such as phenols, cyclopeptide alkaloids, and triterpenoid saponins, according to previous studies on *Ziziphus* genus chemical investigation.

All compounds were distributed in different regions of MS chromatograms (*Figure 2*) and were characterized by comparison of their elution order and HR-MS (full ESI mass spectra and fragmentation patterns) with literature data (*Table 3*). Among phenols, catechins were the most represented, including gallocatechin (peak **2**), epigallocatechin (peak **3**), catechin (peak **6**), and epicatechin (peak **7**), together with their oligomer derivatives (peaks **1**, **4**, and **5**), characterized by fragmentation mass spectra showing an intense negative product ion at *m/z* 125.03. These results agreed with previous studies on *Ziziphus* bark,[21] unlike these compounds were not detected in *Z. lotus* stem bark by Rached et al.,[9] but only in the root bark. The presence of catechin, epicatechin, and procyanidins derivatives, abundant in the stem bark methanolic extract, certainly contributed to its antioxidant property. Several studies have demonstrated the antioxidant potential of catechin, epicatechin, and procyanidins being able to scavenge peroxyl, hydroxyl, superoxide, DPPH, and ABTS radicals.[22,23] It is well-known that tea catechins, which are the main bioactive polyphenols in green tea, significantly suppress TYR activity and melanin synthesis.[24] Therefore, the potential skin whitening properties shown by *Z. lotus* stem bark could be due to the presence of these compounds in the investigated extract.

In addition to catechins, two flavonol glycosides were detected in the extract, myricetin rutinoside

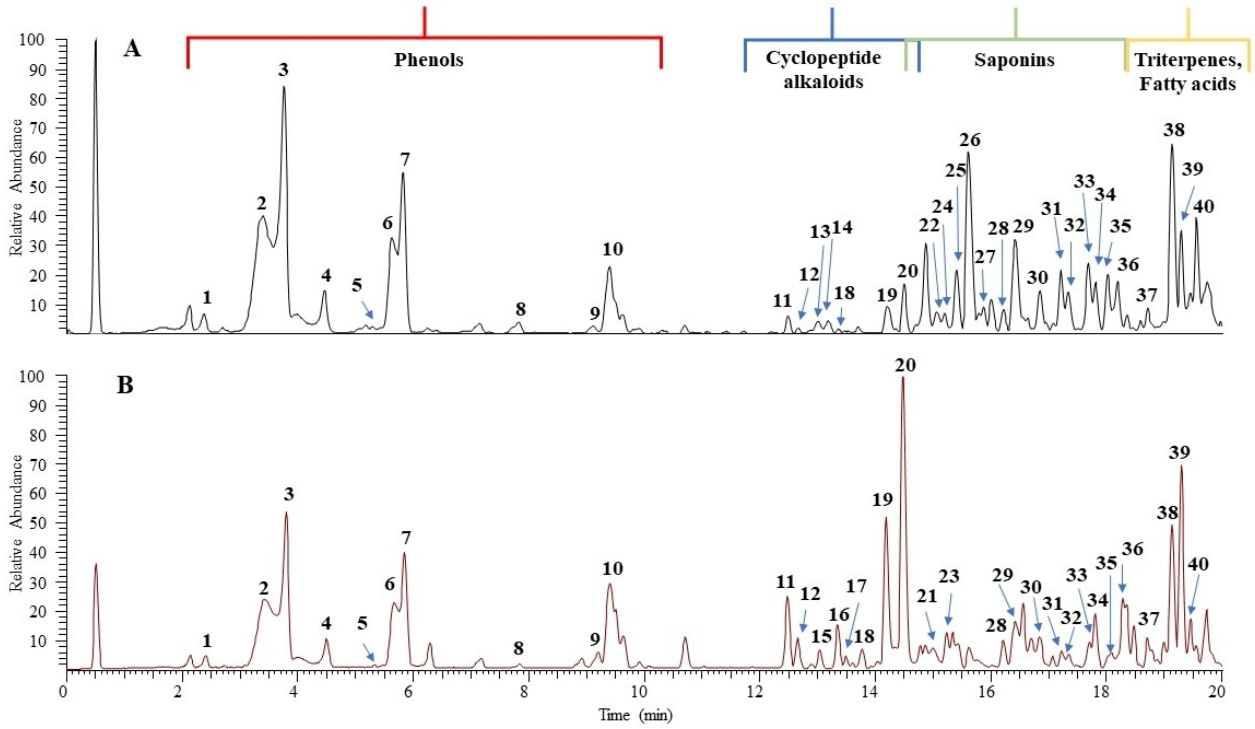

Figure 2. Metabolomic profile of *Z. lotus* stem bark extract obtained by UHPLC-HR-ESI-Orbitrap/MS analysis in both negative (A) and positive (B) ionization modes. Peak numbers correspond to those of *Table 3*.

Table 3. UHPLC-HR-ESI-Orbitrap/MS/MS data (registered in both positive and ion mode) of compounds detected in the *Z. lotus* bark extract. Peaks correspond to those of *Figure 2*.

Peak	Compound[a]	t_R (min)	HR-$[M-H]^-$ (m/z)[b]	HR(−ESI)-MS/MS Product Ions (m/z)[c]	HR-$[M+H]^+$ (m/z)[b]	HR(+ESI)-MS/MS Product Ions (m/z)[c]	Molecular formula	Error (ppm)	Ref.
	Catechins								
1	(*Epi*)Gallocatechin dimer	2.4	609.1255	423.07, 305.07, **125.02**	611.1389	425.09, 287.05, 139.04, **127.04**	$C_{30}H_{26}O_{14}$	+0.82	[9]
2	Gallocatechin	3.4	305.0666	287.06, 219.07, 179.03, **125.02**	307.0807	289.07, 181.05, **139.04**	$C_{15}H_{14}O_7$	−0.33	[9]
3	(*Epi*)gallocatechin	3.8	305.0666	287.06, 219.07, 179.03, **125.02**	307.0807	289.07, 181.05, 181.05, **139.04**	$C_{15}H_{14}O_7$	−0.33	[9]
4	(*Epi*)catechin-(*epi*)gallocatechin	4.5	593.1298	423.07, 305.07, 245.05, **125.03**	595.1437	425.09, 307.08, 247.06, **127.04**	$C_{30}H_{26}O_{13}$	−0.51	[9]
5	Procyanidin dimer (B type)	5.4	577.1356	451.10, 425.09, 289.07,**125.02**	579.1495	427.10, 287.05, 139.04, **127.04**	$C_{30}H_{26}O_{12}$	+0.87	[10]
6	Catechin	5.6	289.0715	245.08, 179.03, 125.02, 109.03	291.0858	207.06, 165.05, **139.04**, 123.04	$C_{15}H_{14}O_6$	−1.04	[9]
7	*Epi*catechin	5.8	289.0719	245.08, 179.03, 125.02, 109.03	291.0859	207.06, 165.05, **139.04**, 123.04	$C_{15}H_{14}O_6$	+0.35	[9]
	Flavonol glycosides								
8	Myricetin rutinoside	7.8	625.1413	317.03, **316.02**, 271.03	627.1552	**319.04**, 273.04, 245.04	$C_{27}H_{30}O_{17}$	+0.48	[35]
9	Quercetin 3-*O*-rutinoside (rutin)	9.1	609.1466	301.03, **300.03**, 271.03	611.1603	465.10, **303.05**, 285.01	$C_{27}H_{30}O_{16}$	+0.82	[25]
	Lignans								
10	Lyoniresinol glucoside	9.4	627.2297 617.2009 *581.2239	419.17, 373.13, 233.09, 153.06	583.2380	249.11, 115.05	$C_{28}H_{38}O_{13}$	−0.17	[25]
	Cyclopeptides								
11	Lotusine F	12.5	519.2613	358.17, 289.12, **155.08**, 148.04, 122.04, 93.03	521.2757	408.19, 299.06, **134.10**, 91.05	$C_{29}H_{36}N_4O_5$	0.00	[5]
12	*N*-methyllotusine F	12.7	533.2764	358.17, 289.12, **155.08**, 148.04, 122.04, 93.03	535.2912	402.40, 299.06, **148.11**, 91.05	$C_{30}H_{38}N_4O_5$	−0.94	
15	*N*-methylphenylalanine-hydroxy-lotusine G	13.0	618.3301	**358.17**, 263.14, 155.08, 122.04	620.3439	261.16, 233.16, **134.10**, 91.05	$C_{34}H_{45}N_5O_6$	+0.65	
16	*N,N*-dimethylphenylalanine-hydroxy-lotusine G	13.4	632.3449	**358.17**, 263.14, 155.08, 122.04	634.3596	360.19, 275.17, **148.11**, 91.05	$C_{35}H_{47}N_5O_6$	−0.79	
17	*N*-methylphenylalanine-lotusine G	13.5	602.3351	–	604.3484	261.16, 233.16, **134.10**, 91.05, 86.10	$C_{34}H_{45}N_5O_5$	+0.50	
18	Lotusine C/*N,N*-dimethylphenylalanine-lotusine G	13.8	616.3504		618.3639	275.17, 247.18, **148.11**, 100.11, 91.05	$C_{35}H_{47}N_5O_5$	0.00	[5]
19	*N*-desmethyl-lotusine E	14.2	632.3456	**358.17**, 263.14, 155.08, 122.04	634.3591	360.19, 275.17, 247.18, **100.11**, 58.06	$C_{35}H_{47}N_5O_6$	+0.32	
20	Lotusine E	14.5	646.3614	**358.17**, 289.12, 155.08, 122.04, 93.03	648.3748	360.19, 289.19, 261.20, **114.13**, 72.08	$C_{36}H_{49}N_5O_6$	+0.62	[5]
21	Cyclopeptide alkaloid	15.0	664.3501	–	666.3641	531.30, 273.16, **148.11**, 114.13, 91.05	–	–	–
23	Lotusine E isomer	15.2	646.3614	358.17, **155.08**, 122.04, 86.71	648.3748	289.19, 261.20, **114.13**, 72.08	$C_{36}H_{49}N_5O_6$	+0.62	[5]
	Saponins		$[M-H]^-$ $[M-Cl]^-$ $[M+HCOO]^-$		$[M+H]^+$ $[2\,M+H]^{++}$				
13	Sulfated saponin	13.0	1071.4708 – –	1039.45, 939.39, 897.38, 490.76, **96.96**	–	–	–	–	–
14	Sulfated saponin	13.2	1071.4694 – –	969.40, 771.31, 689.24, 565.11, 419.05, **96.96**	–	–	–	–	–
22	Lotogenin pentasaccharide (2 hexoses, 1 pentose, 1 deoxyhexose)	15.1	1091.5655 *1127.5417 1137.5704	1091.57, 949.47, 817.42, 655.37, 509.31, 347.26	1093.5778 *546.8006	473.36, 455.35, 437.34	$C_{53}H_{88}O_{23}$	+1.01	
24	Lotoside III	15.2	943.5147 979.5049 *989.5333	655.37, 509.31, 347.26	945.5321 –	455.35, 437.34	$C_{48}H_{80}O_{20}$	0.00	[31]
25	Lotoside I or lotoside II	15.4	959.5234 995.4946 *1005.5283	959.52, 817.42, 797.47, 655.37, 651.41, 509.31, 347.26	961.5369 *480.7798	663.95, 781.84, 473.36, 455.35, 437.34	$C_{48}H_{80}O_{19}$	+1.35	[3]
26	Sulfated saponin (**25**+sulfate)	15.6	1039.4792 – –	897.38, **96.96**,79.96	1041.5119 *521.7612	-	$C_{48}H_{80}O_{22}S$	+0.29	
27	Sulfated saponin malonyl glycoside (**26** + malonyl)	15.9	1125.4799 – –	1081.49, 1039.48, 939.39, 897.38, 655.37, 509.31, 347.26, **96.96**, 79.96	1126.5101 *563.7587	964.48, 455.35, 437.34	$C_{51}H_{82}O_{25}S$	+0.53	
28	Jujubogenin pentasaccharide (3 hexoses, 1 pentose, 1 deoxyhexose)	16.2	1235.6044 1271.5837 *1281.6121	1235.61, 1103.56, 779.46, 633.41	1237.6212 *618.8217	763.46, 617.40, 455.35, 437.34	$C_{59}H_{96}O_{27}$	−1.78	
29	Jujuboside A	16.4	1205.5930 1241.5731 1251.6012	1205.60, 1073.55, 911.51, 749.45	1207.6105 *603.8163	733.46, 587.39, 455.35, 437.34	$C_{58}H_{94}O_{26}$	−2.57	[3]

Table 3. (cont.)

Peak	Compound[a]	t_R (min)	HR-[M−H]$^-$ (*m/z*)[b]	HR(−ESI)-MS/MS Product Ions (*m/z*)[c]	HR-[M+H]$^+$ (*m/z*)[b]	HR(+ESI)-MS/MS Product Ions (*m/z*)[c]	Molecular formula	Error (ppm)	Ref.
30	Jujuboside C	16.9	1073.5524 1109.5310 1119.5593	1073.55, 749.45, 481.51	1075.5673 *537.5973	733.46, 587.39, 455.35, 437.34	$C_{53}H_{86}O_{22}$	−1.30	[3]
31	Sulfated saponin (**32** + pentose)	17.0	1153.5109 – –	1021.47, 771.08, 681.15, 373.04, **96.96**, 79.96	– –	–	$C_{53}H_{86}O_{25}S$	+0.26	
32	3-*O*-α-L-rhamnopyranosyl-(1→2)-[(4-sulfo)-β-D-glucopyranosyl-(1→3)]-β-D-galactopyranosyl-(20*R*,22*R*) 16β,22:16α,30-diepoxydammar-24-ene-3β,20-diol	17.2	1021.4683 – –	636.66, **96.96**, 79.96	1023.4814 *511.7529	779.45, 617.40, 455.35, 437.34	$C_{48}H_{78}O_{21}S$	0.00	[7]
33	Sulfated saponin malonyl glycoside (**32** + malonyl)	17.3	1107.4684 – –	1063.48,1021.47, 779.45, 633.41, 96.96, 79.96	1109.4827 *554.7529	978.73, 749.3, 617.40, 455.35, 437.34	$C_{51}H_{80}O_{24}S$	−0.36	
34	Sulfated saponin, isomer of **32**	17.7	1021.4683 – –	966.53, 770.96, 626.45, **96.96**, 79.96	1023.4815.	617.40, 455.35, 437.34	$C_{47}H_{76}O_{20}S$	0.00	
35	Sulfated saponin, malonyl glycoside isomer of **33**	17.8	1107.4693 – –	1063.48,1021.47, 779.45, 633.41, **96.96**, 79.96	1109.4827	770.96, 490.68, 473.35	$C_{51}H_{80}O_{24}S$	+0.45	
36	3-*O*-α-L-rhamnopyranosyl-(1→2)-[(4-sulfo)-β-D-glucopyranosyl-(1→3)]-α-L-arabinopyranosyl jujubogenin	18.0	991.4584 – –	771.17, 647.83, **96.96**, 79.96	993.4712 *496.7475	749.44, 587.39, 455.35, 437.34	$C_{47}H_{76}O_{20}S$	+0.61	[7]
37	Sulfated saponin malonyl glycoside (**36** + malonyl)	18.2	1077.4587 – –	1033.47, 991.46, 859.32, 627.00, **96.96**, 79.96	1079.4718 *539.7477	835.44, 587.39, 455.35	$C_{50}H_{78}O_{23}S$	+0.46	
	Triterpenes								
39	Ceanothic acid	19.1	485.3271 – –	423.33, 455.32, 364.74, 167.42	487.3415 –	469.33, **441.33**, 451.32, 423.33, 237.15, 203.17	$C_{300}H_{46}O_5$	−0.21	[26]
	Fatty acids								
38	Hydroxy octadecatrienoic acid	18.7	293.2123 – –	**275.20**, 257.19, 231.21, 171.10, 195.14, 121.10	277.2159 [M+H-H_2O]$^+$	259.21, 235.17, 135.12, 121.10, 107.09, 93.07	$C_{18}H_{30}O_3$	+0.34	[34]
40	Hydroxy octadecadienoic acid	19.3	295.2278 – –	277.21, 259.21, 233.23, 185.11, 171.10	279.1589 [M+H-H_2O]$^+$	261.22, 243.21, 149.02, 137.13, 121.03	$C_{18}H_{32}O_3$	−0.34	[26]

[a] Compounds were tentatively identified based on MS data; [b] The asterisk indicates the fragmented parent ions. [c] The base ion peak is shown in bold.

CHEMISTRY & BIODIVERSITY

(peak **8**) and quercetin rutinoside (peak **9**, probably rutin), as deduced by the loss in the MS/MS experiments of the disaccharide chain of rutinose, generating product ions corresponding to the aglycones quercetin (*m/z* at 301.03 and 303.05) and myricetin (*m/z* 317.03 and 319.04) in the negative and the positive ion mode, respectively. Flavonol glycosides were extensively reported in *Ziziphus* fruits[25] and leaves,[26] rarely in the bark.[27,28] Regarding the identification of rutin in wild jujube extract, a computational simulation showed that this compound was able to block the oxidation of L-DOPA binding with TYR enzyme.[29] For this reason, the inhibitory effect of *Z. lotus* extract on TYR could be attributed to the synergic effect of various bioactive compounds such as catechins and flavonol glycosides.

In addition, a lignan glucoside was identified as lyoniresinol glucoside (peak **10**), previously reported in the bark of *Z. rugosa* Lam.[28]

Cyclopeptide alkaloids are considered constituents of taxonomic significance in Rhamnaceae, such as in *Ziziphus* genus. They presented a macrocyclic 13-, 14-, or 15-membered ring structure consisting of a styrylamine unit and two/three amino acids and, in some cases, one/two *N*-methyl or *N*,*N*-dimethyl amino acid residues.[30] In the literature, the presence of jubanine-A type (13-membered ring) and amphibine-B type (14-membered ring) alkaloids was reported in the root bark of Tunisian *Z. lotus* [4-6], while in the leaves of Jordan *Z. lotus* frangulanine type (14-membered ring) alkaloids were reported.[8] LC/MS analyses of herein investigated *Z. lotus* stem bark extract led to the detection of 10 cyclopeptide alkaloids (peaks **11**, **12**, **15**-**21**, and **23**), identified by analyzing mass spectra registered in both ESI positive and negative ionization mode. Mass spectrometry data of peaks **11** and **20** agreed with those of lotusine F and E, jubanine-A type alkaloids previously isolated from the root bark of *Z. lotus*.[5] In addition, other detected cyclopeptide alkaloids were not previously reported, thus the chemical structure was herein proposed based on HR-MS data. The full MS of peak **12** showed a protonated molecular ion $[M+H]^+$ at *m/z* 535.2912, suggesting the presence of an additional methyl group respect to **11** ($[M+H]^+$ at *m/z* 519.2613), due to a *N*,*N*-dimethylphenylalanine residue instead of *N*-methylphenylalanine, as confirmed by the base ion peak at *m/z* 148.11 instead of 134.10 in the mass fragmentation spectra, respectively. Similarly, peak **19** ($[M+H]^+$ at *m/z* 634.3591) showed a product ion at *m/z* 100.11 as base peak, suggesting the presence of a *N*-methylleucine instead of *N*,*N*-dimethylleucine compared to lotusine E (peak **20**, $[M+H]^+$ at *m/z* 648.3748). Peak **23** showed the same protonated molecular ion of **20** at *m/z* 648.3746 and the same fragmentation pathway, suggesting **23** and **20** as isomers having *N*,*N*-dimethylleucine and *N*,*N*-dimethylisoleucine (ion base peak at *m/z* 114.13) as terminal amino acid residue, respectively. Full and mass fragmentation spectra of peak **18** are consistent with the structure of lotusine C, but these data are also in agreement with a lotusine G derivative having as terminal amino acid a residue of *N*,*N*-dimethylphenylalanine (product ion at *m/z* at 148.11). Lotusine G was previously isolated from *Z. lotus* root bark.[6] Peaks **15**, **16**, and **17** appeared to be compounds structurally correlated to **18**. Indeed, peak **17** differed from **18** for having a *N*-methylphenylalanine residue instead of *N*,*N*-dimethylphenylalanine (product ion at *m/z* 134.10), while **15** showed 16 u more, leading to suppose a -OH substituent on the styrylamine unit, such as demonstrated by fragment ions at *m/z* 358.17 also found in the MS/MS of lotusines E and F. Similarly, peak **16** seems to have a hydroxystyrylamine unit and *N*,*N*-dimethylphenylalanine as terminal amino acid. These hypothesized structures need to be confirmed by compounds isolation and characterization by NMR techniques.

Triterpenoid saponins are typical constituents of *Ziziphus* genus. According to literature evidence, the LC/MS analyses of *Z. lotus* stem bark extract led to the tentative identification of different dammarane saponins, most of them mainly identified as monodesmosides having jujubogenin or lotogenin as aglycones through MS analyses registered in both positive and negative ionization modes. Interestingly, the full mass spectra registered in the positive ionization mode showed both $[M+H]^+$ and doubly charged $[M+2H]^{++}$ protonated molecular ions, whose fragmentation generated diagnostic product ions at *m/z* 473.36 (dehydrated lotogenin) and 455.35 (dehydrated jujubogenin/jujubasaponin IV) due to the aglycone moiety, together with other fragments due to the losses of hexose (−162 u), deoxyhexose (−146), and pentose (−132 u) residues. In the MS registered in the negative ion mode, product ions corresponding to deprotonated ($[M-H]^-$) and adduct molecular ions ($[M+HCOO]^-$ and ($[M+Cl]^-$) were observed (*Table 3*). Furthermore, several sulfated saponins were detected, as deduced by the presence of a base ion peak at *m/z* 96.96 in the ESI-MS/MS registered in the negative ionization mode.

Based on literature data, peak **25** ($[M-H]^-$ at *m/z* 959.5234, $[M+H]^+$ at *m/z* 961.5369) could be identi-

fied as either lotoside I or lotoside II, two lotogenin trisaccharides differing for a glucose residue instead of galactose, previously isolated from *Z. lotus* root bark.[3] Compared to **25**, peak **24** showed a trisaccharide chain having one deoxyhexose instead of a hexose, thus suggesting annotation as lotoside III, previously reported in *Z. spina-christi* (L.) Desf. leaves,[31] even though based on MS data it is no possible to define if the saccharide chain was linear (as in lotoside I and II) or branched (as in lotoside III). Compared to **25**, peak **22** displayed one more pentose unit suggesting a pentasaccharide derivative of lotogenin.

Peaks **29** and **30**, were tentatively identified as jujuboside A and jujuboside C, a penta- and a tetrasaccharide of jujubogenin, respectively, previously isolated from the root bark of *Z. lotus*.[3] Compared to **29**, peak **28** showed a hexose instead of a pentose in the saccharide chain. Peaks **32** and **36** were sulfated saponins having a trisaccharide chain linked to jujubonine and jujubasaponin IV aglycones, respectively. Both saponins were isolated in a previous work from the leaves of *Z. lotus*.[7]

Other saponins showed very characteristic fragmentation in the negative ionization mode with product ions due to the loss from the parent ion $[M-H]^-$ of 86 u neutral fragment, attributed to the presence of a malonyl moiety. In particular, peak **33** ($[M-H]^-$ at *m/z* 1107.4684) appeared to have the same structure of **32** ($[M-H]^-$ at *m/z* 1021.4683), except for the acyl group. Similarly, peaks **26** ($[M-H]^-$ at *m/z* 1039.4792), **35** ($[M-H]^-$ at *m/z* 1107.4693), and **37** ($[M-H]^-$ at *m/z* 1077.4587) showed in addition a malonyl unit, compared to peaks **27** ($[M-H]^-$ at *m/z* 1125.4799), **34** ($[M-H]^-$ at *m/z* 1021.4683), and **36** ($[M-H]^-$ at *m/z* 991.4584), respectively, since their molecular weight differ for 86 u. Furthermore, all couple of saponins were very close in terms of retention time. Dammarane saponins were reported having acyl groups such as acetyl, malonyl, and hydroxymethylglutaryl (HMG) linked to the aglycones,[32] but based on previous studies on *Ziziphus* genus, the malonyl is most probably located on the sugar units. Indeed, a malonyl glycoside jujubogenin derivative, named 6‴-*O*-malonyl-Ziziphus saponin, was isolated from *Z. mauritiana* Lam.,[33] while another christinin A2, having a residue of 6-*O*-malonyl-glucopyranose in the saccharide chain linked to the jujubogenin aglycone, was isolated from *Z. spina-christi*.[31] Thus, saponins **27**, **33**, **35**, and **37** were annotated as malonyl glycosides of saponins **26**, **32**, **34**, and **36**, respectively.

Finally, peak **39** was identified as ceanothic acid, a pentacyclic triterpene typical of *Ziziphus* genus,[26] while peaks **38** and **40** were attributed to oxidized fatty acids.[26,34]

Conclusion

In this study, the metabolomic analysis of *Z. lotus* stem bark was investigated by UHPLC-HR-ESI-Orbitrap/MS showing different chemical classes including phenols, cyclopeptide alkaloids, and triterpenoid saponins, confirming its complex and specific chemical composition. Furthermore, the investigated extract showed good antioxidant and tyrosinase inhibitory activities, so that it could be considered a rich source of compounds to be used against free radical related disease such as skin aging and any other common health problems. Several studies have demonstrated the direct correlation between the antioxidant activity and phenolic content. This activity is mainly due to the redox properties of phenolic compounds, which play an important role in neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Besides, as the rich phytocomplex investigated revealed the presence of other bioactive compounds, such as cyclopeptide alkaloids and triterpenoid saponins, their involvement in the whole biological activity cannot be excluded.

In conclusion, this article aims to highlight that the compounds identified from the stem bark of *Z. lotus* could be exploited as potential candidates for developing natural antioxidants in addition to the treatment of hyperpigmentation disorders.

Experimental Section

Solvents and Reagents

Analytical grade methanol for extraction, was purchased from Merck (Germany). UHPLC grade methanol, formic acid, and water were purchased from Romil-Deltek (Italy). For antioxidant assays, Folin–Ciocalteu solution, sodium carbonate (Na_2CO_3), gallic acid, aluminium chloride ($AlCl_3$), sodium acetate (CH_3COONa), ammonium ferrous sulfate, ferrozine, ethylenediaminetetraacetic acid (EDTA), ascorbic acid,2,2-diphenyl-1-picrylhydrazyl (DPPH), mushroom tyrosinase (333 U/mL), L-tyrosine, 3,4-Dihydroxy-L-phenylalanine (L-DOPA), dimethyl sulfoxide (DMSO), and kojic acid were purchased from Merck Life Science S.r.l. (Italy).

Plant Material and Extract Preparation

The stem bark was collected from different plants of *Z. lotus* ($n=5$) growing in the place of Addaura (the northern slopes of Monte Pellegrino, Palermo, Italy (GPS data: 38°11′24.6″N; 13°20′51.6″E), on calcareous lithosol near the sea (February 2020). Plant material identity was confirmed by Prof. F.M. Raimondo of the PLANTA/Center for Research, Documentation and Training (Palermo, Italy). Voucher specimens are stored in the Mediterranean Herbarium (PAL-Gr). After collecting, all the stem barks from the five specimens were combined together and then oven dried at 40 °C. Then, dried parts were grinded using a laboratory mill and the obtained powder was stored in dark at room temperature. To prepare methanolic extract, maceration technique was used: 100 g were stirred with 600 mL of methanol (×3) for 72 h under stirring at room temperature. The obtained extract was evaporated under vacuum at 40 °C after being filtered (yield 19.94%). The extract was stored at +4 °C until further analysis.

Determination of Total Phenolic Content

For determination of total phenolic content, 100 μL of methanol sample solution (1 mg/mL), 2000 μL distilled water, 200 μL Folin–Ciocalteu reagent were mixed. After 3 min, 1000 μL Na_2CO_3 (15%) were added to the mixture and then incubated in the dark at room temperature for 1 h.[36] The absorbance was measured at 765 nm against a blank solution (which contained 100 μL of methanol instead of sample) by a spectrophotometer (Shimadzu UV-1601, Japan). The total polyphenolic content was expressed as gallic acid equivalents (mg GAE/g extract) calculated by a calibration curve). (60–600 μg/mL) ($y=0.0026x-0.0624$ $R^2=0.9920$). All tests were performed in triplicates.

Determination of Total Flavonoids

For determination of total flavonoids, 100 μL of methanolic extract (1 mg/mL), 400 μL of distilled water, and 30 μL $NaNO_2$ (5%) were added to a glass cuvette. Then, 30 μL of $AlCl_3$ (10%) were added after 5 min, 200 μL NaOH (1 M) and 240 μL distilled water were added after 6 min. The solution was mixed and its absorbance was measured at 510 nm against a blank solution (which contained 100 μL of methanol instead of sample).[36]

Results were expressed as rutin equivalents (mg RE/g extract) using a calibration curve). (20–500 μg/mL) ($y=0.0018x-0.0356$ $R^2=0.9903$). All tests were performed in triplicates.

UHPLC-HR-ESI-Orbitrap/MS Analyses

The methanolic extract of *Z. lotus* was analyzed by means of UHPLC-HR-ESI-MS. The LC/MS system was composed by a Vanquish Flex Binary pump LC and a Q Exactive Plus MS, Orbitrap-based FT-MS system (Thermo Fischer Scientific Inc., Germany). Before UHPLC/MS analyses, the methanolic extract was partitioned between BuOH and H_2O to remove sugars and very polar primary metabolites. The BuOH extract was finally dissolved in methanol (2 mg/mL) and injected (5 μL) on a C-18 Kinetex® Biphenyl column (100× 2.1 mm, 2.6 μm particle size) provided of a Security Guard TM Ultra Cartridge (Phenomenex, Italy). The elution was performed at a flow rate 0.5 mL/min, by using formic acid in MeOH 0.1% v/v (solvent A) and formic acid in H_2O 0.1% v/v (solvent B) and developing a linear solvent gradient from 5 to 80% A within 20 min. The autosampler and column oven temperatures were maintained at 4 and 35 °C, respectively. HR mass spectra were acquired in a scan range of *m/z* 270–1500 in both ESI negative and positive ionization modes, operating in full (70000 resolution, 220 ms maximum injection time) and data dependent-MS/MS scan (17500 resolution, 60 ms maximum injection time). Ionization parameters were optimized as previously reported.[37]

Antioxidant Activity

The antioxidant activity of wild jujube methanolic extract was estimated by three different tests: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay, Fe^{3+} reducing power (FRAP), and Fe^{2+} chelating measurement.

DPPH Radical-Scavenging Assay

For DPPH radical-scavenging assay, aliquots (0.5 mL) of extract (250, 500, 1000 μg/mL) were mixed with 3 mL of a freshly prepared DPPH methanol solution (0.1 mM). The reaction mixture was shaken vigorously and kept in the dark at room temperature for 30 min; then, the absorbance was measured at wavelength of 515 nm. Ascorbic acid was used as reference standard, and results were reported as ascorbic acid equivalents

(mg AAE/g extract).[36] All tests were performed in triplicates.

Fe^{3+} Reducing Antioxidant Power (FRAP)

FRAP was determined according to the method of Uysal et al.[38] Sample solution (1 mg/mL; 0.1 mL) was added to premixed FRAP reagent (2 mL) containing acetate buffer (0.3 M, pH 3.6), 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) (10 mM) in 40 mM HCl and ferric chloride (20 mM) in a ratio of 10 : 1 : 1 (v/v/v). Then, the sample absorbance was read at 593 nm after a 30 min incubation at room temperature. FRAP activity was expressed as milligrams of Trolox equivalents (mg TE/g extract). All tests were performed in triplicates.

Fe^{2+} Chelating Activity

Fe^{2+} chelating activity was determined according to the method by Dinis et al.[39] with some modifications, by measuring the ferrous iron-ferrozine complex absorbance at 562 nm. Aliquots (150 μL) of *Ziziphus* extract (250—1000 μg/mL) were incubated with 1050 μL of sodium acetate buffer (5 %, pH 6.9), 150 μL of Fe^{2+} (ammonium ferrous sulfate, 20 μM) and 150 μL of ferrozine (100 μM). The mixture was shaken and left at room temperature for 10 min. The absorbance of the resulting solution was measured at 562 nm. The capability to chelate ferrous iron was calculated using the equation below:

$$\text{Chelating activity } (\%) = \frac{A_0 - A_s}{A_0} \times 100$$

where A_0 is the absorbance of the control and A_S the absorbance of the sample solution.

As reference standard ethylenediaminetetraacetic acid (EDTA) was employed and results were reported as milligrams of EDTA equivalents (mg EDTA/g extract). All tests were performed in triplicates.

Mushroom Tyrosinase Inhibition Assay

Tyrosinase inhibitory activity assay was performed according to the method described by Smeriglio et al.[12,13] The inhibitory effects of *Z. lotus* methanolic extract on TYR were assessed evaluating both the monophenolase and diphenolase activity of the enzyme. Aliquots (50 μL) of extract (50–500 μg/mL) were mixed with 500 μL of substrate, L-DOPA or L-tyrosine (1.25 mM), and 900 μL of sodium acetate buffer (50 mM, pH 6.8). The reaction mixture was allowed to incubate for 10 min at 25 °C, thereafter, 50 μL of an aqueous solution of mushroom tyrosinase (333 U/mL) was added. The optical density of the reaction mixture was recorded at 475 nm until 30 or 1 min, for monophenolase or diphenolase activity, respectively. DMSO and kojic acid (1–50 μg/mL) were used as negative and positive controls.

The tyrosinase inhibitory activity was calculated according to the following equation:

$$\text{Inhibition } (\%) = \frac{A - B}{A} \times 100$$

where A is the absorbance at 475 nm with tyrosinase and substrate (L-DOPA or L-tyrosine) without test sample and B is the absorbance at 475 with tyrosinase, substrate (L-DOPA or L-tyrosine) and test sample. Results were expressed as the concentration capable of inhibiting the 50 % of the enzyme activity (IC_{50}). All tests were performed in triplicates.

Data Evaluation

A Pearson linear correlation was carried to evaluate the relationship between total phenolic and flavonoid content and the antioxidant activities using the SigmaPlot 12.0 software. Statistical significance was considered at $p < 0.05$.

Acknowledgements

Open Access Funding provided by Universita in Università degli Studi di Messina within the CRUI-CARE Agreement. Open Access Funding provided by Universita degli Studi di Messina within the CRUI-CARE Agreement.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contribution Statement

Anna Cacciola: Methodology, Investigation, Data curation, Writing original draft. Valeria D'Angelo: Validation

and Data curation. Francesco Maria Raimondo: Resources, Data curation. Maria Paola Germanò: Methodology, Investigation, Data curation, Writing – review & editing. Alessandra Braca: Supervision, Data curation, Writing – review & editing. Marinella De Leo: Methodology, Investigation, Data curation, Writing – review & editing.

References

[1] E. El Maaiden, Y. El Kharrassic, N. A. S. Qarahd, A. K. Essamadia, K. Moustaidb, B. Nassera, 'Genus *Ziziphus*: A comprehensive review on ethnopharmacological, phytochemical and pharmacological properties', *J. Ethnopharmacol.* **2020**, *259*, 112950.

[2] J. Fakchich, M. Elachi, 'An overview on ethnobotanico-pharmacological studies carried out in Morocco, from 1991 to 2015: Systematic review (part 1)', *J. Ethnopharmacol.* **2020**, *267*, 113–200.

[3] J. H. Renault, K. Ghedira, P. Thepenier, C. Lavaud, M. Zeches-Hanrot, L. Le Men-Olivier, 'Dammarane saponins from *Zizyphus lotus*', *Phytochemistry* **1997**, *44*, 1321–1327.

[4] K. Ghedira, R. Chemli, B. Richard, J.-M. Nuzillard, M. Zeches, L. Le Men-Olivier, 'Two cyclopeptide alkaloids from *Zizyphus lotus*', *Phytochemistry* **1993**, *32*, 1591–1594.

[5] K. Ghedira, R. Chemli, C. Caron, J.-M. Nuzillard, M. Zeches, L. Le Men-Olivier, 'Four cyclopeptide alkaloids from *Zizyphus lotus*', *Phytochemistry* **1995**, *38*, 767–772.

[6] G. Le Crouéour, P. Thépenier, B. Richard, C. Petermann, K. Ghédira, M. Zèches-Hanrot, 'Lotusine G: a new cyclopeptide alkaloid from *Zizyphus lotus*', *Fitoterapia* **2002**, *73*, 63–68.

[7] A. Maciuk, C. Lavaud, P. Thépenier, M. J. Jacquier, K. Ghédira, M. Zèches-Hanrot, 'Four new dammarane saponins from *Zizyphus lotus*', *J. Nat. Prod.* **2004**, *67*, 1639–1643.

[8] M. Abu-Zarga, S. Sabri, A. al-Aboudi, M. S. Ajaz, N. Sultana, Atta-Ur-Rahman, 'New cyclopeptide alkaloids from *Ziziphus lotus*', *J. Nat. Prod.* **1995**, *58*, 504–511.

[9] W. Rached, L. Barros, B. E. C. Ziani, M. Bennaceur, R. C. Calhelha, S. A. Heleno, M. J. Alves, A. Marouff, I. C. F. R. Ferreira, 'HPLC-DAD-ESI-MS/MS screening of phytochemical compounds and the bioactive properties of different plant parts of *Zizyphus lotus* (L.) Desf.', *Food Funct.* **2019**, *10*, 5898.

[10] I. Marmouzi, M. Kharbach, M. El Jemli, A. Bouyahya, Y. Cherrah, A. Bouklouze, Y. V. Heyden, M. El Abbes Faouzi, 'Antidiabetic, dermatoprotective, antioxidant and chemical functionalities in *Zizyphus lotus* leaves and fruits', *Ind. Crops Prod.* **2019**, *132*, 134–139.

[11] A. Sanchez-Ferrer, J. N. Rodriguez-Lopez, F. Garcia-Canovas, F. Garcia-Carmona, 'Tyrosinase: a comprehensive review of its mechanism', *Biochim. Biophys. Acta* **1995**, *1247*, 1–11.

[12] A. Smeriglio, V. D'Angelo, M. Denaro, D. Trombetta, M. P. Germanò, 'The hull of ripe pistachio nuts (*Pistacia vera* L.) as a source of new promising melanogenesis inhibitors', *Plant Foods Hum. Nutr.* **2021**, *76*, 111–117.

[13] A. Smeriglio, V. D'Angelo, M. Denaro, D. Trombetta, M. P. Germanò, 'Polyphenol characterization, antioxidant and skin whitening properties of *Alnus cordata* stem bark', *Chem. Biodiversity* **2019**, *16*, e1900314.

[14] S. Abdoul-Azize, 'Potential benefits of jujube (*Zizyphus lotus* L.) bioactive compounds for nutrition and health', *J. Nutr. Metab.* **2016**, *16*, 1–13.

[15] L. A. Abderrahim, K. Tai, C. A. Abderrahim, 'Assessment of the antimicrobial and antioxidant activities of *Ziziphus lotus* and *Peganum harmala*', *Iran. J. Sci. Technol., Trans. A: Sci.* **2017**, *43*, 409–414.

[16] O. O. Olajuyigbe, A. J. Afolayan, 'Phenolic content and antioxidant property of the bark extracts of *Ziziphus mucronata* Willd. subsp. *mucronata* Willd', *BMC Complementary Altern. Med.* **2011**, *11*, 130–138.

[17] M. K. M. Akanda, A. H. M. N. Hasan, 'Characterization of pharmacological properties of methanolic seed and stem bark extracts of *Ziziphus mauritiana* (BAU Kul) using *in vitro* and *in vivo* animal (Swiss albino male mice) model', *Clin. Phytosci.* **2021**, *7*, 8–20.

[18] M. A. Soobrattee, V. S. Neergheen, A. Luximon-Ramma, O. I. Aruoma, T. Bahorun, 'Phenolics as potential antioxidant therapeutic agents: Mechanism and actions', *Mutat. Res., Fundam., Mol. Mech. Mutagen.* **2005**, *579*, 200–213.

[19] B. Aggarwal, P. Sharma, H. S. Lamba, 'Assessment of phytochemical and *in vitro* antioxidant potential of *Zizyphus nummularia* stem bark', *Int. J. Pharma Res. Health Sci.* **2018**, *6*, 2502–2506.

[20] S. Parvez, M. Kang, H. S. Chung, H. Bae, 'Naturally occurring tyrosinase inhibitors: mechanism and applications in skin health cosmetics and agriculture industries', *Phytother. Res.* **2007**, *21*, 805–816.

[21] A. Malik, Z. A. Kuliev, Y. A. Akhmedov, A. D. Vdovin, N. D. Abdullaev, 'Proanthocyanidins of *Ziziphus jujuba*', *Chem. Nat. Compd.* **1997**, *33*, 165–173.

[22] Y. Yilmaz, R. T. Toledo, 'Major flavonoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin, and gallic acid', *J. Agric. Food Chem.* **2004**, *52*, 255–260.

[23] M. Grzesik, K. Naparlo, G. Bartosz, I. Sadowska-Bartosz, 'Antioxidant properties of catechins: comparison with other antioxidants', *Food Chem.* **2018**, *241*, 480–492.

[24] X. Zhang, J. Li, Y. Li, Z. Liu, Y. Lin, J. Huang, 'Anti-melanogenic effects of epigallocatechin 3-gallate (EGCG), epicatechin-3-gallate (ECG) and gallocatechin-3-gallate (GCG) via down regulation of CAMP/CREB /MITF signaling pathway in B16F10 melanoma cells', *Fitoterapia* **2020**, *145*, 104634.

[25] A. M. Pawlowska, F. Camangi, A. Bader, A. Braca, 'Flavonoids of *Zizyphus jujuba* L. and *Zizyphus spina-christi* (L.) Willd (Rhamnaceae) fruits', *Food Chem.* **2009**, *112*, 858–862.

[26] S. T. Sakna, A. Mocan, H. N. Sultani, N. M. El-Fiky, L. A. Wessjohann, M. A. Farag, 'Metabolites profiling of *Ziziphus* leaf taxa via UHPLC/PDA/ESI-MS in relation to their biological activities', *Food Chem.* **2019**, *293*, 233–246.

[27] R. L. Khosa, V. B. Pandey, J. P. Singh, 'Experimental studies on *Zizyphus rugosa* (Lam) bark', *Indian Drugs* **1983**, *20*, 241.

[28] J. Sichaem, T. Aree, K. Lugsanangarm, S. Tippyang, 'Identification of highly potent α-glucosidase inhibitory and antioxidant constituents from *Zizyphus rugosa* bark:

Enzyme kinetic and molecular docking studies with active metabolites', *Pharm. Biol.* **2017**, *55*, 1436–1441.
[29] Y.-X. Si, S.-J. Yin, S. Oh, Z.-J. Wang, S. Ye, L. Yan, J.-M. Yang, Y.-D. Park, J. Lee, G.-Y. Qian, 'An integrated study of tyrosinase inhibition by rutin: Progress using a computational simulation', *J. Biomol. Struct. Dyn.* **2012**, *29*, 999–1012.
[30] N.-H. Tan, J. Zhou, 'Plant cyclopeptides', *Chem. Rev.* **2006**, *106*, 840–895.
[31] A. Bozicevic, M. De Mieri, A. Di Benedetto, F. Gafner, M. Hamburger, 'Dammarane-type saponins from leaves of *Ziziphus spina-christi*', *Phytochemistry* **2017**, *138*, 134–144.
[32] S. C. Mendonça, R. C. Simas, D. L. Reis Simas, S. G. Leitão, G. G. Leitão, 'Mass spectrometry as a tool for the dereplication of saponins from *Ampelozizyphus amazonicus* Ducke bark and wood', *Phytochem. Anal.* **2021**, *32*, 262–282.
[33] C. S. Seri, T. A. Okpekon, P. A. Yao-Kouassi, A. A. Magid, C. Sayagh, L. Voutquenne-Nazabadioko, 'Saponins and flavonoid glycosides from the leaves of *Ziziphus mauritiana* Lam. native of a forest area of Ivory Coast', *Phytochem. Lett.* **2020**, *37*, 5–9.
[34] M. Almeida-Trapp, G. Donizetti de Souza, K. Shekhawat, A. H. Sheikh, A. Mithöfer, H. Hirt, E. Rodrigues-Filho, 'Development, validation, and application of an HPLC/MS/MS method for quantification of oxidized fatty acids in plants', *J. Chromatogr. B* **2021**, *1186*, 123006.
[35] J. C. Andrade, A. R. P. Silva, A. T. L. Santos, M. A. Freitas, J. N. P. Carneiro, M. I. P. Gonçalo, A. de Souza, T. S. Freitas, P. R. V. Ribeiro, E. S. Brito, M. F. B. Morais-Braga, H. D. M. Coutinho, 'UPLC/MS-ESI-QTOF characterization and evaluation of the antibacterial and modulatory antibiotic activity of *Ziziphus joazeiro* Mart. aqueous extracts', *S. Afr. J. Bot.* **2019**, *123*, 105–112.
[36] M. De Leo, A. M. Iannuzzi, M. P. Germanò, V. D'Angelo, F. Camangi, F. Sevi, G. Diretto, N. De Tommasi, A. Braca, 'Comparative chemical analysis of six ancient Italian sweet cherry (*Prunus avium* L.) varieties showing antiangiogenic activity', *Food Chem.* **2021**, *360*, 129999.
[37] L. Flori, M. Macaluso, I. Taglieri, C. Sanmartin, C. Sgherri, M. De Leo, V. Ciccone, S. Donnini, F. Venturi, L. Pistelli, A. Martelli, V. Calderone, L. Testai, A. Zinnai, 'Development of fortified *Citrus* olive oils: from their production to their nutraceutical properties on the cardiovascular system', *Nutrients* **2020**, *12*, 1557.
[38] S. Uysal, G. Zengin, M. Locatelli, M. B. Bahadori, A. Mocan, G. Bellagamba, E. De Luca, A. Mollica, A. Aktumsek, 'Cytotoxic and enzyme inhibitory potential of two *Potentilla* species (*P. speciosa* L. and *P. reptans* Willd.) and their chemical composition', *Front. Pharmacol.* **2017**, *8*, 1–11.
[39] T. C. P. Dinis, V. M. C. Madeira, L. M. Almeida, 'Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers', *Arch. Biochem. Biophys.* **1994**, *315*, 161–169.

Received March 16, 2022
Accepted May 4, 2022

