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ANTIOXIDANT PROPERTIES OF THE NEEDLES OF *TAXUS BACCATA* L. GROWING IN ALGERIA AND CHARACTERIZATION OF THEIR ANTIOXIDANT CONSTITUENTS BY LC–DAD–ESI–MS^n

Mohamed Bekhouche[1*], Abdelkader Morsli[1], Majda Khelifi Slaoui[1], Roukia Benyammi[1], Soumia Krimat[2], Cedric Paris[3], Lakhdar Khelifi[1]

[1]Ecole Nationale Supérieure Agronomique (ENSA-ES1603), Laboratoire de Ressources Génétiques et Biotechnologies (LRGB), El Harrach-Algiers, Algeria.

[2]Laboratoire de molécules bioactives et valorisation de biomasse, ENS-kouba, Alger, Algérie

[3]Ecole Nationale Supérieure d'Agronomie et des Industries Alimentaires, Laboratoire d'Ingénierie des Bio-Molécules, ENSAIA, Nancy, France.

*Corresponding author E-mail: **mohamed.bekhouche@edu.ensa.dz**

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ABSTRACT

Introduction: *Taxus* genus has shown remarkable therapeutic results as a medicinal plant; hence, the present work is conducted to comprehensively characterize polyphenols of the methanol needles extracts from two *Taxus baccata* populations growing in Algeria. **Methods:** The determination of phenolic compound profile of the methanol extracts of the Algerian yew was established for the first time using liquid chromatography with diode-array detection coupled to electrospray ionization tandem mass spectrometry (LC–DAD–ESI–MS^n), while the total phenolic and flavonoid contents as well as the antioxidant activities were measured according to standard protocols. **Results:** Using LC–DAD–ESI–MS^n analysis, 28 compounds including 12 phenolic acids and 16 flavonoids have been identified; three of which have never been previously reported in *Taxus baccata* such as sinapic acid, taxifolin and apigenin. The total phenolic and the total flavonoid contents in *Taxus baccata* showed significant differences ($p<0.05$) according to the populations. The total phenolic and flavonoid contents were relatively greater at Chrea population (TPC = 125.84 ± 4.35 mg GAE/ g dry extract; TFC = 220.1 ± 6.36 mg RE/g dry extract) compared to Tikjda population (TPC = 100.12 ± 0.28 mg GAE/g dry extract; TFC = 166.6 ± 1.73 mg RE/g dry extract). Similarly, the highest antioxidant activity was observed with the methanolic extracts from Chrea population [(DPPH IC50 = 29.44 ± 0.99 µg/ml, ABTS IC50 = 10.94 ± 1.06 µg/ml and FRAP Value = 58.72 ± 0.57 mmol TE/100g DW)]. The antioxidant capacities of the same extract were statistically higher than the synthetic antioxidant BHT. **Conclusion:** The obtained results highlight the importance of *Taxus baccata* needles as a rich source of bioactive compounds that can be exploited in the food and pharmaceutical field.

INTRODUCTION: A large number of human pathologies are favored when the production of free radicals exceeds the antioxidant defense capacities [1]. This oxidative stress state is usually due to excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [1, 2]. These species can damage cells starting with chain chemical reactions such

as peroxidation of lipids or by oxidation of DNA and proteins [3–5]. These chemical reactions cause cellular dysfunction that are involved in various pathologies such as cancer [7, 8], cardiovascular diseases and neurodegenerative diseases [4, 9]. Synthetic antioxidant molecules such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) used to reduce oxidative stress are currently being challenged due to potential toxicological and even carcinogenic risks [9]. Hence, natural antioxidants could be an alternative to reduce oxidative stress [8, 10]. *Taxus baccata* L., which is commonly known as yew, belongs to the *Taxaceae* family and is a traditional medicinal and ornamental plant. It is mainly distributed in Europe, Eastern Asia and North-Western Africa [11, 12]. There are seven closely related species worldwide [13] including *Taxus baccata* L. (yew), which is the only representative in Algeria. In particular, *T. baccata* has been traditionally used as a herbal remedy for the treatment of bronchitis, asthma, diarrhea and epilepsy [14]. Previous studies have reported that *Taxus baccata* has various pharmacological and physiological properties including antimicrobial, anticancer, anti-inflammatory and antioxidants [15, 16]. These activities are attributable to the various bioactive compounds that are present in *Taxus baccata* including phenolic compounds, flavonoids, alkaloids and toxoids [18, 19]. Despite the fact that phenolic compounds were previously reported as constituents of the *Taxaceae* family plants [17, 20–22], only few studies have been carried out on the phenolic composition of *Taxus baccata* [23, 24], and little is known about the phenolic acids and flavonoids of the plant's needles. This is the first study for the identification of phenolic acids and flavonoids of *Taxus baccata* L. growing in Algeria. Therefore, the present research aims to profile the phenolic compounds of yew methanol extracts using liquid chromatography coupled with mass spectrometry (LC–DAD–ESI–MS^n), and to investigate the antioxidant properties in relation to the amounts of total polyphenols in needles methanolic extracts.

MATERIAL AND METHODS

Chemical procurement: 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis3-ethylbenzothiazoline-6-sulphonic acid (ABTS), 2,4,6-tri 2-pyridyl -s-triazine (TPTZ), Iron (III) chloride hexa-hydrate ($FeCl_3$ $6H_20$), ascorbic acid (AA), 2,6-Di-tert-butyl-4 methylphenol (BHT) and 6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid (Trolox) were obtained from Sigma Chemical Co. Methanol and acetonitrile (HPLC grade) were purchased from Aldrich Co. All other solvents and chemicals were of analytical grade.

Collection of plant materials: The fresh needles of *Taxus baccata* L. were collected in July and August 2016 from two regions of Algeria: Chrea National Park (36° 24′ 00″ N, 2° 52′ 00″ E) and Djurdjura National Park Tikjda station (36° 26′ 75″ N, 4° 07′ 40″ E). The plant species was identified by Professor Salima Benhouhou, Department of Plant Sciences, Algerian Higher National Agronomic School. Samples were, then, shade dried at room temperature and ground into fine powder.

Sample preparation: The ground needles 10 grams (g) were mixed using 100 ml methanol. The mixture was stirred for 24 hours with continuous shaking. This extraction process was repeated twice. The mixture was filtered, and the solvent was removed using a rotary evaporator (Büchi R II V-700). The obtained methanol extracts were filtered through 0.22 µm membrane and were stored in dark glass bottles at 4 °C until used for further analyses.

LC–DAD–ESI–MS^n analysis of phenolic compounds: The phenolic compounds present in the methanolic extracts of *T. baccata* were identified using an LC–DAD–ESI–MS^n. The samples were analyzed by HPLC-MS system (Thermo Fisher Scientific, USA) equipped by a binary solvent delivery pump connected to a photodiode array detector (PDA) and a LTQ mass spectrometer equipped with an atmospheric pressure ionization interface operating in electrospray mode (ESI). The samples were separated on a C18 Alltima (150mm * 2.1mm) column (Grace/Alltech, Darmstadt, Germany). The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The samples were eluted using a first linear gradient from 2% to 20% of B for 70 min, and then a second linear gradient from 20% to 80% of B for

30 min. Mass analysis was carried out in ESI positive ion mode (ESI+). Mass spectrometric conditions were as follows: spray voltage was set at 4.5 kV; source gases were set (in arbitrary units min^{-1}) for sheath gas; auxiliary gas and sweep gas at 40, 10 and 10, respectively; capillary temperature was set at 300 °C; capillary voltage at 36 V; tube lens, split lens and front lens voltages at 80 V, - 44 V and - 3.25 V, respectively. Full scan MS spectra (100 to 2000 m/z) and data dependent MS^2 scans for structural investigation were performed on LTQ (Linear Trap Quadripole). Raw data were processed using the XCALIBUR software program (version 2.1). Experimental exact masses and MS^2 fragmentation data were compared to metabolomics Mass Bank: (http://www.massbank.jp and Pubchem Compound:http://pubchem.ncbi.nlm.nih.gov), and other available data from the literature in order to identify the nature of the metabolites.

Determination of total phenolic content (TPC): The total phenolic content of *T. baccata* methanolic extracts was determined according to the Folin–Ciocalteu method [24]. Gallic acid was used as standard and the concentration of total phenolic compounds in the extracts were calculated by standard curve interpolation (y = 0.0098 + 0.0064; R^2 = 0.99). Results were reported as mg gallic acid equivalent per gram of dry extract (mg GAE/g dry extract).

Determination of total flavonoid content (TFC): The total flavonoid content (TFC) of *T. baccata* methanolic extracts was determined photometrically according to the aluminum chloride colorimetric method [25]. The concentration of total flavonoid content was calculated based on a standard curve prepared using rutin (y = 0.001 + 0.0006; R^2 = 0.99), and expressed as milligrams rutin equivalent per gram of dry extract (mg RE/g dry extract).

Antioxidant activity Analysis: DPPH radical-scavenging activity assay: The antioxidant activity of the methanolic extracts of *T. baccata* were determined using the DPPH free radical scavenging assay described by Patra [26] with minor modifications. The test was performed on a 96-well microplate mixing 80 µl of methanolic extract at different concentrations or positive controls (BHT and ascorbic acid) with 220 µl of freshly prepared DPPH solution (0.1 Mm). The reaction mixtures were incubated for 30 minutes in the dark, and the absorbance is measured at 517 nm using a microplate reader (SAFAS, Xenius Monaco, and France). The percentages of inhibition of the DPPH radical were calculated according to the following equation:

DPPH scavenging activity (%) = [(A0 - A1)/A0] x 100.

Where A0 = absorbance of the control and A1 = absorbance of the test extracts.

ABTS free radical-scavenging activity assay:

The ability of the methanolic extracts of *Taxus baccata* to trap free radicals ABTS (2,2'-azino-bis3-ethylbenzothiazoline-6-sulphonic acid) was measured as described by Le Grandois [27] with some modifications. Briefly, in a 96-well microplate, 80 µl volume of the methanolic extract at different concentrations or control (BHT and ascorbic acid) was mixed with 220 µl of freshly prepared ABTS solution (7 Mm). The reaction mixtures were incubated for 15 minutes in the dark, and the absorbance is measured at 734 nm using a microplate reader (SAFAS, Xenius Monaco, France). The test was repeated three times for each concentration. The percentages of inhibition of the ABTS radical were calculated according to the following equation: ABTS radical inhibition (%) = [(A0 - A1)/A0] x 100.

Where A0 = absorbance of the control and A1 = absorbance of the test extracts.

Ferric Reducing Antioxidant Power Assay (FRAP): The total antioxidant power of the methanolic extracts of *Taxus baccata* were measured using the ferric reducing antioxidant power method as described by Youn [28] with some modifications. The FRAP solution was prepared by mixing 25 ml of acetate buffer solution at 300 mM (pH 3.6), 2.5 ml of 10 mM 2,4,6-tri-2-pyridyl -s-triazine solution (TPTZ) and 2.5 ml of 20 mM hexa-hydrated iron (III) chloride solution ($FeCl_3$ $6H_20$) with a mixing ratio of (10:1 v/v). The absorbance was measured at 539 nm against a blank. The trolox was used as the standard solution for calibration. The antioxidant capacity was calculated on the basis of the ability of the sample to reduce ferric

ions from the linear calibration curve and expressed as millimoles trolox equivalents per hundred gram dry weight of sample (µmol trolox E/ g DW). All of the treatment groups were measured in triplicate.

Statistical analysis: The data were expressed as means of three replicate determinations ± standard deviation (SD). One-way analysis of variance (ANOVA) was used and combined with LSD test (Least Significant Difference). The differences were considered to be significant at $P<0.05$. All statistical analyses were performed with SPSS 19.0 for windows.

RESULTS AND DISCUSSION

Identification of phenolic compounds: The phenolic compounds of the methanol needle extracts of the two *Taxus baccata* populations growing in Algeria were established for the first time using a liquid chromatography with diode-array detection coupled to electrospray ionization tandem mass spectrometry (LC–DAD–ESI–MS^n) in positive mode. The methanolic yew extracts showed similar phenolic profiles and the identified compounds are listed in Table 1.

Characterization of phenolic acids: Twelve phenolic acids were identified. The first one (compound (1)), was assigned to quinic acid, showed $[M + H]^+$ peak at m/z 193 at 3.80 min and produced the MS^2 fragments ions at m/z 175 corresponding to the loss of water molecule $[M + H - H2O]^+$ and at m/z 165; corresponding to the loss of a carboxyl group $[M + H - CO2]^+$. The second one (compound (2)) with a retention time 4.14 min produced a MS^2 fragments ions at m/z 147 corresponding to the loss of a carboxyl group $[M + H - CO2]^+$, and it was identified as shikimic acid [29]. The third one (compound (3)), was assigned to Benzoic acid, showed $[M + H]^+$ peak at m/z 123 at 4.28 min and produced the MS^2 fragments ions at m/z 105 corresponding to the loss of water molecule $[M + H - H2O]^+$ and at m/z 95; corresponding to the loss of a carboxyl group $[M + H - CO2]^+$ [29]. The fourth one (compound (4)) with a retention time 5.71 min produced the MS^2 fragments ions at m/z 121 corresponding to the loss of water molecule $[M + H - H2O]^+$. Similarly, compounds 5, 6, 7, 8 and 9 with retention times (t_R) of 10.96, 18.09, 22.11, 27.33 and 29.88 min and an $[M+ H]^+$ at m/z 199, 149, 169, 165 and 171 were identified as syringic acid, cinnamic acid, vanillic acid, *p*-coumaric acid and gallic acid respectively [30]. These compounds have been previously detected in *Taxus baccata* [29]. Compound (10) with a retention time of 36.61 min and $[M + H]^+$ at m/z 225 produced the MS^2 fragments ions at m/z 207 $[M + H - H2O]^+$ and at m/z 181 $[M + H - HCOOH]^+$. Based on the above fragmentation, this compound was identified as sinapic acid (Fig.1). This phenolic acid has been detected for the first time in *T. baccata* L. Compounds 11 and 12 (t_R37.25 and 37.41 min) with pseudomolecular ions $[M +H]^+$ of 183 and 181 were assigned as dihydrocaffeic acid and caffeic acid, respectively, after comparing their retention times and MS/MS fragmentation patterns with those reported in literature [31].

Characterization of flavonoids: Sixteen flavonoids were identified including 2 flavan-3-ols, 1 flavanonol, 8 flavonols, 1 flavones and 4 biflavonoids.

Characterization of flavan-3-ol: Two peaks with identical molecular formula $C_{15}H_{14}O_{6,}$ and an $[M + H]^+$ at m/z 291 were assigned as catechin (13) and epicatechin (14). These compounds were detected at retention times of 33.26 and 44.54 min and produced the MS^2 base peak at m/z 139, corresponding to the loss of a retro Diels–Alder (RDA) fragment $[M + H - 152Da]^+$.

Characterization of flavanonol: One flavanonol was identified. Compound (15) with a retention time of 46.39 min and $[M + H]^+$ at m/z 271 produced the MS^2 fragments ions at m/z 287 $[M + H - H2O]^+$ and at m/z 259 $[M + H - HCOOH]^+$, and it was identified as taxifolin [31]. This compound has been detected for the first time in *Taxus baccata*.

Table 1. Identification of phenolic acids and flavonoids in Algerian *Taxus baccata* L.by HPLC–DAD–ESI–MS^n in positive ionization mode.

No	Identification	Molecular Formula	Rt (min)	M	$[M+H]^+$ (m/z)	HPLC-ESI-MS^n (m/z)	MEC	MET
Phenolic acid								
1	Quinic acid [a]	$C_7H_{12}O_6$	3.80	192	193	193, 175, 165	+	+
2	Shikimic acid [a]	$C_7H_{10}O_5$	4.14	174	175	175, 147, 139	+	+
3	Benzoic acid [a]	$C_7H_6O_2$	4.51	122	123	123, 105, 95	+	+
4	*p*-hydroxybenzoic acid [a]	$C_7H_6O_3$	5.71	138	139	139, 121, 111	+	+
5	Syringic acid [a]	$C_9H_{10}O_5$	10.96	198	199	199, 181, 153	+	+
6	Cinnamic acid [a]	$C_9H_8O_2$	18.09	148	149	149, 131, 121	+	+
7	Vanillic acid	$C_8H_8O_4$	22.11	168	169	169, 151, 123	+	+
8	*p*-coumaric acid [a]	$C_9H_8O_3$	27.33	164	165	165, 147, 123	+	+
9	Gallic acid	$C_7H_6O_5$	29.88	170	171	171, 153, 125	+	+
10	Sinapic acid [c]	$C_{11}H_{12}O_5$	36.61	224	225	225, 207, 179	+	+
11	Dihydrocaffeic acid [a]	$C_9H_{10}O_4$	37.25	182	183	183,165, 147	+	+
12	Caffeic acid [a]	$C_9H_8O_4$	37.41	180	181	181, 163, 145	+	+
Flavan-3-ol								
13	Catechin [a,b]	$C_{15}H_{14}O_6$	33.26	290	291	291, 165, 139	+	+
14	Epicatechin [a,b]	$C_{15}H_{14}O_6$	44.59	290	291	291, 165, 139	+	+
Flavananol								
15	Taxifolin [b]	$C_{30}H_{26}O_{12}$	46.39	304	305	305, 287, 259	+	+
Flavanol								
16	Myricetin-3-*O*-rutinoside [a,b]	$C_{27}H_{30}O_{17}$	59.25	626	627	319	+	+
17	Myricetin [a,b]	$C_{15}H_{10}O_8$	60.35	318	319	319, 301, 273	+	+
18	Quercetin-3-*O*-rutinoside[a,b]	$C_{21}H_{20}O_{12}$	67.06	610	611	303	+	+
19	Quercetin-7-*O*-glucoside [a,b]	$C_{27}H_{30}O_{16}$	68.64	464	465	303	+	+
20	Quercetin [a,b]	$C_{15}H_{10}O_7$	69.11	302	303	303, 153, 137	+	+
21	Kaempferol 3-*O*-rutinoside[a,b]	$C_{27}H_{30}O_{15}$	74.04	594	595	287	+	+
22	Kaempferol 7-*O*-glucoside[a,b]	$C_{21}H_{20}O_{11}$	74.53	448	449	287	+	+
23	kaempferol [a,b]	$C_{15}H_{10}O_6$	75.59	286	287	287, 269, 241	+	+
Flavone								
24	Apigenin [c]	$C_{21}H_{20}O_{10}$	76.53	432	433	271, 243, 225	+	+
Biflavonoid								
25	Amentoflavone [a,b]	$C_{30}H_{18}O_{10}$	89.02	538	539	403, 421, 377	+	+
26	Bilobetin[a]	$C_{31}H_{20}O_{10}$	90.17	552	553	553, 435, 391	+	+
27	Ginkgetin [a]	$C_{32}H_{20}O_{10}$	94.26	566	567	567,449, 417	+	+
28	Sciadopitysin [a,b]	$C_{33}H_{24}O_{10}$	99.70	580	581	581, 549, 449	+	+

Rt: retention time; M: Molecular Weight; a: Compounds Previously reported in *T.baccata* ; b: Compounds Previously reported in other *Taxus* species; c : Compounds not previously reported in *T. baccata*;MEC: Methanolic extracts from Chrea; MET: Methanolic extract from Tikjda.

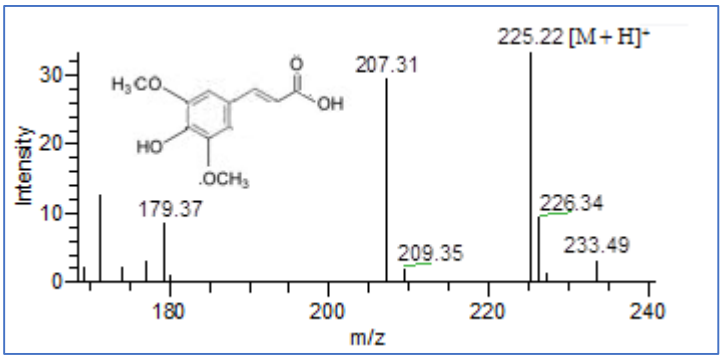

Fig.1. Mass spectra and chemical structure of Sinapic acid detected in Algerian *Taxus baccata* by LC–DAD–ESI–MS^n

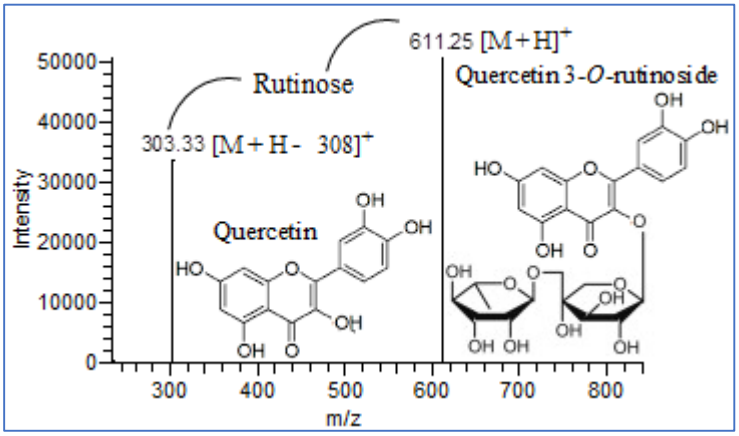

Fig.2. Mass spectra and chemical structures of quercetin 3-*O*-rutinoside detected in Algerian *Taxus baccata* by LC–DAD–ESI–MS^n

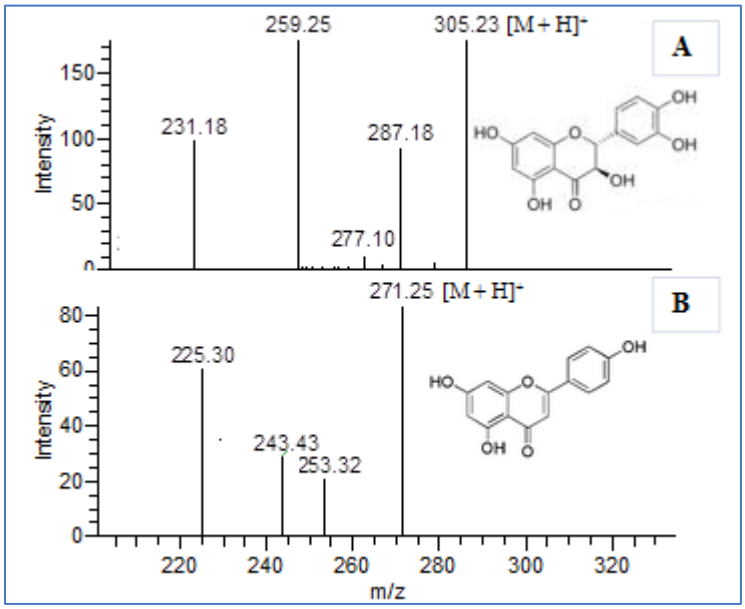

Fig.3.Mass spectra and chemical structures of new phenolic compounds detected in Algerian *Taxus baccata* by LC–DAD–ESI–MS^n. A) Taxifolin, B) Apigenin.

Table 2. Total phenol and flavonoid contents and antioxidant activities in the methanolic extracts from the two populations of *Taxus baccata* L. growing in Algeria.

Extracts & standards	TPC (mgGAE/g)	TFC (mg RE/g)	DPPH IC50 (µg/ml)	ABTS IC50 (µg/ml)	FRAP (mmol TE/100g DW)
MEC	125.84 ± 4.35^{a}	220.1 ± 6.36^{a}	29.44 ± 0.99^{a}	10.94 ± 1.06^{b}	58.72 ± 0.57^{b}
MET	100.12 ± 0.28^{b}	166.6 ± 1.73^{b}	61.70 ± 3.15^{c}	14.24 ± 1.27^{c}	33.22 ± 0.17^{c}
BHT	-	-	53.50 ± 3.39^{b}	15.26 ± 1.12^{c}	52.58 ± 4.27^{b}
AA	-	-	28.15 ± 084^{a}	2.31 ± 0.02^{a}	507.06 ± 9.26^{a}

Results are expressed as mean ± SD. Values with no letters in common are significantly different ($p< 0.05$) for each index. DW: Dry weight ofsample. IC_{50}: The concentration of sample and standard that can inhibit 50% of DPPH and ABTS scavenging capacity, MEC: Methanolic extracts from Chrea; MET: Methanolic extract from Tikjda.

Characterization of flavonol: In this study, eight flavonols were identified. Compound (16) (t_R 59.21 min) was identified as myricetin 3-*O*-rutinoside with a pseudomolecular ion $[M+H]^+$of 627. It produced the MS^2 base peak at m/z 319 corresponding to myricetin after the neutral loss of one molecule of rutinose ($[M+H-308]^+$). Similarly, compounds 18 and 21 with t_R 67.06 and 74.04 min were assigned as quercetin 3-*O*-rutinoside (Fig.2) and kaempferol 3-*O*-rutinoside [33,34]. Compounds 17, 20 and 23 (t_R 60.35, 69.11 and 75.59 min) with pseudomolecular ions $[M+H]^+$ of 319, 303 and 287 were assigned as myricetin, quercetin and kaempferol, respectively, after comparing their retention times and MS/MS fragmentation patterns with those reported in literature [33, 36]. Compound (19) was identified as quercetin 7-*O*-glucoside with a pseudomolecular ion $[M+H]^+$ of 465. It produced the MS^2 base peak at m/z 303 corresponding to quercetin after the neutral loss of one molecule of glucose ($[M+H-162]^+$). Similarly, compound 22 with t_R 74.533 min was assigned as kaempferol 7-*O*-glucoside [23].

Characterization of flavones: Compound (24) with a retention time of 76.35 min and $[M+H]^+$ at m/z 271 produced the MS^2 fragments ions at m/z 253 $[M+H-H2O]^+$, at m/z 243 $[M+H-CO]^+$ and at m/z 225 $[M+H-HCOOH]^+$. According to the above fragmentation, this compound was identified as apigenin (M 270). This flavone has been identified for the first time in *T. baccata* (Fig.3).

Biflavonoid: In addition, four bioflavonoids were identified. Compounds 25, 26, 27 and 28 with molecular formula $C_{30}H_{18}O_{10}$, $C_{31}H_{20}O_{10}$, $C_{33}H_{24}O_{10}$ and $C_{21}H_{21}O_{11,}$ and $[M+H]^+$ at m/z 139, at m/z 553, at m/z 567 and at m/z 581 were detected at retention times of 89.02, 90.17, 94.26 and 99.70 min, respectively. These compounds were assigned as amentoflavone , bilobetin , ginkgetin and sciadopitysin, respectively, after comparing their MS/MS fragmentation patterns with those reported in literature [33, 37, 38].

Determination of total phenolic and total flavonoid content: The concentrations of the total phenols and the total flavonoids of *Taxus baccata* methanolic extracts were determined by the Folin-Ciocalteu and the Aluminum Chloride methods, respectively. The results showed that the *T.baccata* methanolic extracts were found to contain very high amounts of phenols and flavonoids (Table 2). The total phenolic and flavonoid contents were significantly ($p<0.05$) greater in the methanolic extract from Chrea (TPC= 125.84 ± 4.35 mg GAE/g dry extract; TFC = 220.1 ± 6.36 mg RE/g dry extract) compared to the methanolic extracts from Tikjda (TPC= 100.12 ± 0.28 mg GAE/g dry extract; TFC = 166.6 ± 1.73 mg RE/g dry extract). Probably, the differences in the total phenolic and flavonoid contents could be attributed to genetic variation, distinct environmental, geographic origins, climatic conditions, and plant populations [37, 38]. Very few studies have been conducted to measure the total phenol and flavonoid contents of *T. baccata* needles extracts. For example, Guleria [39], found a total phenol content of 69.96 ± 2.73 mg GAE/g in the methanol extract of the Indian *Taxus baccata*. Milutinović [17], reported that the total phenolic and the total flavonoid contents of the methanolic extracts from the Serbian *Taxus baccata* were 92.13 ± 0.84 mg GAE/g dry extract and 161.98 ± 1.02 mg RE/g dry extract,

respectively. The results of our investigation are superior to those mentioned above. As a result, it confirms the richness of Algerian *Taxus baccata* L. needle extracts in flavonoids, especially the population of Chrea.

Antioxidant activity analysis:

The antioxidant properties of the methanolic extracts of the two *Taxus baccata* populations and the positive controls (BHT and AA) have been determined by the three well-known method (DPPH, ABTS and FRAP) due to their stability, reproducibility and precision [1, 40–42]. The results are shown in Table 2. Statistical analysis indicated that there was significant difference ($P < 0.05$) between the two populations for their antioxidant activity. The highest antioxidant activities were obtained from the methanolic extracts from Chrea population (DPPH IC_{50} = 29.44 ± 0.99 µg/ml, ABTS IC_{50} = 10.94 ± 1.06 µg/ml and FRAP Value= 58.72 ± 0.57 mmol TE/100g DW). The antioxidant capacities of the same extract were statistically higher than the synthetic antioxidant BHT (DPPH IC_{50} = 53.50 ± 3.39 µg/ml, ABTS IC_{50} = 15.26 ± 1.12 µg/ml and FRAP Value= 52.58 ± 4.27 mmol TE/100g DW). However, all antioxidant activities recorded in the methanolic extracts of *Taxus baccata* were significantly ($P < 0.05$) lower than that of the positive control (Ascorbic acid). From literature, the DPPH, ABTS and FRAP are reactive towards most antioxidants including phenolic compounds [43–45]. Many phenolic compounds such as phenolic acids and flavonoids are found to be strong antioxidants effectively scavenging the DPPH and ABTS radicals and reducing metal ions (FRAP) because of their phenolics hydroxyl groups [45, 47]. Our study revealed the presence of twelve phenolic acids and sixteen flavonoids in the methanolic extracts of Algerian *T. baccata* which might play an important role in absorbing and neutralizing free radicals or reducing metal ions. In this context, the results of our investigation are in accordance with the studies published by Guleria [39] and Milutinović [17] which mentioned that the *Taxus baccata* needles extracts have strong antioxidant properties, acting as free radical scavengers and metal ion reducing agents.

CONCLUSION

In conclusion, our study investigates for the first time the phenolic compound profile and evaluates total phenolic and flavonoid contents in addition to the antioxidant activities of needles extracts obtained from the two *Taxus baccata* L. populations growing in Algeria. Our findings revealed that the methanolic extracts were found to have very high phenolic and flavonoid contents. The analysis of the methanol extracts by LC–DAD–ESI–MS^n showed the presence of 28 phenolic compounds including 12 phenolic acids and 16 flavonoids confirming the medicinal interest of this plant. These bioactive compounds have very valuable antioxidant properties, acting as free radical scavengers and metal ion reducing agents. From these results, it was concluded that the *T. baccata* extracts could be considered as a potential source of natural bioactive molecules that can be exploited in the food and pharmaceutical field.

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Authors' contributions:

Mohamed Bekhouche collected the samples, performed all the experiments, and drafting the manuscript with Abdelkader Morsli, Roukia Benyammi and Soumia Krimat. The authors read the manuscript and approved the final version.

Conflicts of interest:

The authors declared no conflict of interest.

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