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Flavonoid profile and antioxidant properties of Algerian common yew (*Taxus baccata* L.)

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Abstract

Background: In humans, various diseases are associated with the accumulation of free radicals. The antioxidants can scavenge free radicals and reduce their impact; thus, the search for effective natural antioxidants of plant origin is indispensable. The present study aims to determine, for the first time, the flavonoid compounds profile and to investigate the free radical scavenging and antioxidant properties of the methanolic extract of *Taxus baccata* L. from Algeria.

Methods: The determination of the flavonoid compound profile of the methanolic extract of *Taxus baccata* L. was established using high-performance liquid chromatography with diode-array detection coupled to electrospray ionization tandem mass spectrometry (HPLC–DAD–ESI–MS/MS). The total flavonoid content (TFC) was performed according to the aluminum chloride colorimetric method, while the free radical scavenging and antioxidant activities were carried out using three methods, namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay, 2,2'-azino-bis3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical assay and ferric reducing antioxidant power (FRAP) Assay.

Results: A total of 26 compounds including flavon-3-ols, flavanonols, flavones, flavonols and bioflavonoids were characterized and identifiedusing HPLC–DAD–ESI–MS/MS analysis, five were reported for the first time such as taxifolin, apigenin, apigenin 7-*O*-glucoside, isorhamnetin 3-*O*-rutinoside and robustaflavone. The plant extract exhibited high total flavonoid content (TFC = 204.26 ± 6.02 mg RE/g dry extract) which corresponded to its strong radical scavenging activities [(DPPH IC_{50} = 35.31 ± 0.29 μg/ml and ABTS IC_{50} = 8.27 ± 0.52 μg/ml)] as compared to the synthetic antioxidant BHT [(DPPH IC_{50} = 78.96 ± 5.70 μg/ml and ABTS IC_{50} = 13.56 ± 0.06 μg/ml)]. However, the methanolic extract of *T. baccata* showed the lowest ferric reducing ability as compared to the positive controls (BHT, BHA, ascorbic acid, trolox and quercetin).

Conclusion: Our results imply that the *Taxus Baccata* L. might be a potential source for the isolation of natural antioxidant compounds.

Keywords: Antioxidants, Flavonoids, Scavenging activity, HPLC–MS/MS, *Taxus baccata* L

Background

Taxus genus, commonly known as yew, is a gymnosperm in the *Taxaceae* family which is mainly distributed in North America, Europe, Eastern Asia and North-Western Africa.There are nine species of small trees or shrubs [1]. One of the species, Taxus baccata L (Common yew), is represented in Algeria. Based on current knowledge on the distribution of species of Mediterranean flora, Algerian yews would be closely related to Italo-Balkan yews. However, some authors have noted significant differences in yews from Algeria, even proposing a new taxon at the intraspecific level, such as *Taxus baccata* var. microphylla [2]. Until now, there is no phylogenetic study confirmed this taxon, described exclusively based on morphological characters.

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Otherwise,*Taxus baccata* has been traditionally used as a herbal remedy for the treatment of asthma, bronchitis, epilepsy and diarrhea [3].However, it also has therapeutic properties, being indicated for its antioxidant, anticancer, anti-microbial and anti-inflammatory actions. Besides, previous studies carried out with needle extracts of *Taxus baccata* L. showed their effect on inhibiting the migration of cancer cells as well as on DNA protection [4, 5].

Processes such as mitochondrial respiration can generate free radicals as products of metabolism [6, 7]. Nevertheless, excess production might play a role in the pathophysiology of many disease conditions including cardiovascular diseases, neurodegenerative disorders, diabetes and cancer [8, 9]. Antioxidants are substances that delay or inhibit cellular damage mainly through their free radical scavenging activity [10]. In recent years, there has been increasing interest in the discovery of natural antioxidant substances as they can protect the human body from free radicals and delay the development of some chronic diseases [8, 11]. Natural antioxidants are widely distributed in food and medicinal plants. These natural antioxidants, especially phenolic compounds and flavonoids, exhibit a wide range of biological effects [12].

Flavonoids are an essential group of phenolic substances extensively studied for their possible beneficial effects on human health [13, 14]. These natural products are now considered as an indispensable component in a variety of medicinal, pharmaceutical, and cosmetic applications [14, 15]. This is attributed to their anti-inflammatory, antioxidative, anticarcinogenic and antimutagenic properties along with its capacity to modulate key cellular enzyme function [15, 16]. Flavonoids are diverse bioactive compounds that can be classified into various classes such as flavones, flavonols, flavan-3-ols, flavanonols, isoflavones and bioflavonoids [17, 18]. Furthermore, flavonoids can be extracted from a diverse range of sources ranging from microorganisms to higher plants [19].

Some research papers have been published regarding the chemical composition and biological activities of bark and heartwood of *Taxus baccata* [20, 21], but only a few studies have focused on evaluating antioxidant activity and identifying flavonoid compounds from the needles [22, 23]. Moreover, to our knowledge there is no research about flavonoid compounds of the Algerian yew species.

The present research aims to profile the flavonoid compounds of the methanolic extract of the Algerian yew needles using high-performance liquid chromatography coupled with mass spectrometry (HPLC–ESI–MS/MS) and to evaluate the free-radical scavenging and antioxidant activities of its methanolic extract.

Materials and methods
Chemicals and reagents
2,2'-azino-bis3-ethylbenzothiazoline-6sulphonic acid (ABTS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tri 2-pyridyl -s-triazine (TPTZ), Iron (III) chloride hexa-hydrate ($FeCl_3$ $6H_2O$), rutin, quercetin, ascorbic acid, 6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid (Trolox), butylated-hydroxytoluene (BHT) and butylatedhydroxyanisole (BHA), methanol of analytical grade and acetonitrile of HPLC grade were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Plant material
The fresh needles of *Taxus baccata* L. were collected in July 2016 from El Hamma Test Garden (36° 44′ 53″ N-3° 04′ 34″ E). The plant species were authenticated in the Botanical Department, Algerian Higher National Agronomic School, where a voucher specimen (HNLA/FA/P510) of the plant has been lodged in the herbarium of the school. The samples were air-dried at room temperature in the shade and then powdered.

Preparation of methanolic extract
The extraction method was carried out as described by Gutiérrez et al., [23] with some modifications. Briefly, 20 g (g) of powdered *T. baccata* were extracted in 100 mL methanol for 24 h at room temperature with frequent agitation. The extraction process was repeated twice. the collected filtrate was then concentrated to dryness in a rotary evaporator (Büchi R II V-700) under reduced pressure at temperature (35–40 °C) to obtain dry extract (5.3 g). Finally, dry extract was diluted in methanol to 1 mg/mL, passed through 0.45 μm membrane filter (Millipore Filter) and stored at 4 °C until used for further analyses.

Determination of total flavonoid content (TFC)
The total flavonoid content of *T. baccata* methanolic extract was determined by Aluminum chloride colorimetric method [24]. Briefly, 500 μL aliquot of the methanolic extract (1 mg/mL) was mixed with 2500 μL distilled water and 150 μL sodium nitrite (2.5%: m/v). After 5 min, 150 μL aluminium chloride (10%: m/v) was added to the mixture. Subsequently, 2000 μL of sodium hydroxide (1 M) and 200 μL of distilled water were added after 6 min. The mixture was shaken and the absorbance was read at 510 nm after 15 min incubation in dark at room temperature using an UV–visible spectrophotometer (SAFAS, Xenius Monaco, France). Total flavonoid compounds was evaluated from a calibration curve established with standard rutin (y = 0.001 + 0.0006; R2 = 0.99) at concentrations from 0 to 400 μg/mL prepared under the same experimental conditions. Results were reported

as mg Rutin equivalent per gram of dry extract (mg RE/g dry extract). Analyses were performed in triplicate.

HPLC–DAD–ESI–MS/MS analysis of flavonoid compounds
HPLC–DAD analysis
The HPLC–DAD method proposed by Larbat et al., [25] was used for identified flavonoid compounds present in the methanolic extract of *Taxus baccata* L. The HPLC apparatus (Thermo Fisher Scientific, USA) consisted of a binary solvent delivery pump connected to a photodiode array detector (PDA) was used for acquiring chromatograms. The flow rate was set at 200 µL/min and injection volume was 5 µL. Sample was prepared in methanol, at a concentration of 1 mg/mL and filtered through 0.45 µm membrane filter (Millipore Filter). The mobile phases consisted of water modified with formic acid (0.1%) for A and acetonitrile modified with formic acid (0.1%) for B. The studies were carried out on a C18 Alltima (150 mm * 2.1 mm) column (Grace/Alltech, Darmstadt, Germany). Sample was 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.

MS/MS analysis
The MS analysis was performed on LTQ mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with an atmospheric pressure ionization interface operating in electrospray mode (ESI) coupled to the LC apparatus for mass spectrometric identification of characteristic peaks. Mass analysis was first carried out in ESI positive ion mode (ESI^+), and secondly in ESI negative ion mode (ESI^-). Mass spectrometric conditions were as follows for ESI^+ mode: spray voltage 4.5 kV, capillary temperature 300 °C, sheath gas flow rate at 40, sweep gas flow rate at 10, capillary voltage at 36 V. split lens and front lens voltages at 80 V,—44 V and—3.25 V, respectively. For ESI^- mode, MS conditions were unchanged except ion optics parameters which were automatically adapted as follows: 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, Pubchem Compound: http://pubchem.ncbi.nlm.nih.gov), and other available data from the literature to identify the nature of the metabolites.

DPPH free radical-scavenging assay
The method of Patra et al., [26], with some modifications was used to determine the DPPH Free radical scavenging ability of the methanol extract of *T. baccata*. The test was performed on a 96-well microplate by mixing 80 µL of the methanolic extract or positive controls (BHT, BHA, ascorbic acid, trolox and quercetin prepared in methanol) at different concentrations [from 1 µg/mL to 100 µg/mL] with 220 µL of freshly prepared DPPH solution (0.1 mM). The reaction mixture was incubated in the dark for 30 min and the absorbance was read at 517 nm using a microplate reader (SAFAS, Xenius Monaco, and France). The test was repeated three times for each concentration. The DPPH scavenging capacity of the extract was calculated using the following equation [27]:

DPPH radical scavenging activity (%) = [(A_C—A_S)/A_C] × 100.

Where, A_C = absorbance of the control, and A_S = absorbance of the sample.

The IC_{50} value is the concentration of the sample required to scavenge 50% of DPPH free radical. It was calculated from the non-linear regression analysis of DPPH radical scavenging activity against various concentrations of a sample [28].

ABTS free radical scavenging activity assay
The ABTS radical scavenging activity of the methanol extract of *T. baccata* was measured as described by Le Grandois et al., [29] with modifications. Briefly, in a 96-well microplate, 80 µL volume of the methanolic extract or positive controls (BHT, BHA, ascorbic acid, trolox and quercetin prepared in methanol) at different concentrations [from 1 µg/mL to 100 µg/mL] was mixed with 220 µl of freshly prepared ABTS solution (7 mM). The reaction mixtures were incubated for 15 min in the dark and the absorbance is measured at 734 nm using a microplate reader. The test was repeated three times for each concentration. The ABTS scavenging capacity of the extract was determined using the following formula [30]:

ABTS radical scavenging activity (%) = [(A_C—A_S)/A_C] × 100.

where A_C = absorbance of the control and A_S = absorbance of the sample. The scavenging activity of the sample was expressed as IC_{50} (µg/mL).

Ferric Reducing Antioxidant Power Assay (FRAP)
The antioxidant capacity of the methanolic extract of *Taxus baccata* was measured using the ferric reducing antioxidant power method as described by Youn [31] 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

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-s-triazine solution (TPTZ) and 2.5 mL of 20 mM hexahydrated iron (III) chloride solution (FeCl$_3$ 6H$_2$0) with a mixing ratio of (10:1 v/v). The methanolic extract or positive controls (BHT, BHA, ascorbic acid, trolox and quercetin prepared in water) were tested at different concentrations [from 1 μg/mL to 100 μg/mL]. 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 based on the ability of the sample to reduce ferric ions from the linear calibration curve and expressed as micromoles trolox equivalents per gram of dry extract (μmol TE/ g dry extract). All of the tests were measured in triplicate.

Statistical analysis of data

The results were expressed as mean ± standard deviation (SD) of three replicates. One-way analysis of variance (ANOVA) and least significant difference (LSD) test were used to determine significant differences between the sample and the references. The differences were considered to be significant at $P < 0.05$. All statistical analyses were performed using the SPSS 19.0 software package.

Results and discussion

LC–DAD–ESI–MS/MS analysis of flavonoid compounds

In this study, we identified for the first time the flavonoid compounds of the methanolic extract of Algerian *T. baccata* using HPLC–DAD–ESI–MS/MS in positive and negative ion mode. A total of 26 compounds including flavon-3-ols, flavanonols, flavones, flavonols and biflavonoids were identified and characterized based on their LC retention times, mass data generated by LTQ-MS, MS/MS fragmentation patterns, or by comparison with literature data. The identified flavonoids are listed in Table 1, where the compounds are numbered according to their retention times in the obtained chromatograms (Fig. 1).

Characterization of flavan-3-ols

Six flavan-3-ols were detected (Table 1). The first flavan-3-ol (Compound (1)) with molecular formula C$_{30}$H$_{26}$O$_{12}$ and pseudomolecular ion [M − H]$^-$of 577, was assigned as B-type proanthocyanidin (PA) with (epi)catechin monomeric units. This compound generated the MS^2 base peak at m/z 407 [M − H − 152 Da − 18 Da]$^-$ by the loss of a retro Diels–Alder (RDA) fragment (152 Da) followed by the loss of a water molecule (18 Da); secondary peak.

at m/z 451 [M − H − 126 Da]$^-$ from heterocyclic ring fission (HRF), and at m/z 289 [(epi) catechin − H]$^-$ originate from a quinonemethide (QM) fragment (Fig. 2). Based on the above fragmentation, this proanthocyanidin was identified as (epi)catechin-(4,8)-(epi)catechin [32].

The second flavan-3-ol (Compound (2)) was determined as dimeric B-type proanthocyanidin, with molecular formula C$_{30}$H$_{26}$O$_{13}$ and pseudomolecular ion [M − H]$^-$of 593. It produced daughter ions at m/z 407 [M − H − 168 Da – 18 Da]$^-$ by the loss of a retro Diels–Alder (RDA) fragment (168 Da) followed by the loss of a water molecule (18 Da), at m/z 307 ([(epi)gallocatechin − H])$^-$ originate from a quinonemethide (QM) fragment and at m/z 289 [(epi) catechin − H]$^-$ originate from a (QM) fragment (Fig. 1). According to the above fragmentation, this compound was identified as (epi)catechin-(4,8)-(epi)gallocatechin [33]. The third flavan-3-ol (compound (3)) was assigned to dimeric B-type proanthocyanidin with (epi)gallocatechin monomeric unit. It displayed [M − H]$^-$ peak at m/z 609 at 34.07 min and produced the MS^2 base peak at m/z 423 ([M −H −168 Da − 18 Da]$^-$) by the loss of an RDA fragment (168 Da) followed by the loss of a water molecule (18 Da); secondary peaks at m/z 305 ([(epi)gallocatechin–H]) originate from a QM fragment and at 441 ([M−H–168 Da]$^-$) from an RDA fragment (Fig. 2). The presence of a QM fragment at m/z 305 showed that the top and base units are (epi) gallocatechin From the preceding arguments, this proanthocyanidin must have two (epi)gallocatechin units [33].

The fourth flavan-3-ol (Compound (4)) was identified as (Epi)gallocatechin, showed [M − H]$^-$ peak at m/z 305 at 34.32 min and generated the MS^2 fragments ions at m/z 261, 221, 219 and 179, corresponding to the loss of a 44 Da fragment (C02), a 84 Da fragment (C$_4$H$_4$O$_2$), a 86 Da fragment (C$_4$H$_6$O$_2$) and a 126 Da fragment (C$_6$H$_6$O$_3$), respectively [33].

Two isomer flavan-3-ols (Compounds 5 and 8) with identical molecular formula C$_{15}$H$_{14}$O$_6$ and [M − H]$^-$ peak at m/z 289, were identified as catechin and epicatechin, respectively. These compounds were detected at retention times of 35.17 and 45.92 min and produced the MS^2 base peak at m/z 245, corresponding to the loss of a carboxyl group [M − H − CO2]$^-$ [32].

Characterization of flavanonol

One flavanonol (Compound (9)) was detected and identified as taxifolin (Table 1), with molecular formula C$_{30}$H$_{26}$O$_{12}$ and [M + H] + peak at m/z 305. It generated the MS^2 fragments ions at m/z 287 corresponding to the loss of water molecule [M + H − H2O]$^+$ and at m/z 259 corresponding to the loss of a carboxyl group [M + H − HCOOH]$^+$ (Fig. 0.2) [34]. This flavanonol has been detected for the first time in *Taxus baccata* L.

Characterization of flavone

Two flavones were detected. The first flavone (Compound (10)) was identified as apigenin 7-*O*-glucoside, with pseudomolecular ion [M + H]$^+$ of 433. It generated the MS^2

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Table 1 Identification of flavonoid compounds in *Taxus baccata* L. by HPLC–ESI–MS/MS in positive and negative ionization mode

N°	Proposed compounds	Molecular formula	Rt (min)	M	$[M+H]^+$/ $[M-H]^-$ (m/z)	HPLC–ESI–MS/MS (m/z)
Flavon-3-ol						
1	(Epi)catechin-(4,8)-(epi)catechin[a,b]	$C_{30}H_{26}O_{12}$	31.00	578	577	451, **407**, 289
2	(Epi)catechin-(4,8)-(epi)gallocatechin[a,b]	$C_{30}H_{26}O_{13}$	33.66	594	593	407, 305, 289
3	(Epi)gallocatechin-(4,8′)-(epi)gallocatechin[a,b]	$C_{30}H_{26}O_{14}$	34.07	610	609	**423**, 441, 305
4	(Epi)gallocatechin[a,b]	$C_{15}H_{14}O_7$	34.32	306	305	261, 221, **219**, 178.97
5	Catechin[a,b]	$C_{15}H_{14}O_6$	35.17	290	289	289, **245**, 205, 179
8	Epicatechin[a,b]	$C_{15}H_{14}O_6$	45.92	290	289	289, **245**, 205, 179
Flavanonol						
9	Taxifolin[b]	$C_{30}H_{26}O_{12}$	46.38	304	305	**305**, 287, 259
Flavone						
10	Apigenin 7-*O*-glucoside[c]	$C_{21}H_{20}O_{10}$	51.18	432	433	**271**
11	Apigenin[c]	$C_{21}H_{20}O_{10}$	56.51	270	271	**271**, 243, 225
Flavanol						
12	Myricetin-3-*O*-rutinoside[a,b]	$C_{27}H_{30}O_{17}$	62.91	626	627	**319**
13	Myricetin[a,b]	$C_{15}H_{10}O_8$	63.44	318	319	319, 301, **273**
14	Quercetin-3-*O*-rutinoside[a,b]	$C_{21}H_{20}O_{12}$	66.97	610	611	**303**
15	Quercetin-7-*O*-glucoside [a,b]	$C_{27}H_{30}O_{16}$	69.25	464	465	**303**
16	Quercetin [a,b]	$C_{15}H_{10}O_7$	70.90	302	303	**303**, 285, 257, 165
17	Kaempferol 3-*O*-rutinoside[a,b]	$C_{27}H_{30}O_{15}$	74.02	594	595	**287**
18	kaempferol 7-*O*-glucoside[a,b]	$C_{21}H_{20}O_{11}$	74.39	448	449	**287**
19	kaempferol[a,b]	$C_{15}H_{10}O_6$	75.57	286	287	**287**, 269, 240.9
20	Isorhamnetin 3-O-rutinoside[c]	$C_{28}H_{32}O_{16}$	76.04	624	625	**317**
Biflavonoid						
21	Amentoflavone[a,b]	$C_{30}H_{18}O_{10}$	87.78	538	539	539, **403**, 421, 377
22	Robustaflavone[c]	$C_{30}H_{18}O_{10}$	88.77	538	539	413, **387**, 270
23	Bilobetin[a]	$C_{31}H_{20}O_{10}$	90.39	552	553	553, 435,391, 297
25	Ginkgetin[a]	$C_{32}H_{20}O_{10}$	94.47	566	567	567,449, 417, 405
26	Sciadopitysin[a,b]	$C_{33}H_{24}O_{10}$	99.83	580	581	581, **549,** 449, 415
Unidentified compounds						
6	n.d	-	38.59	155	156	155, 113, 109, 95
7	n.d	-	39.77	155	156	**155**, 113, 109, 95
24	n.d	-	93.26	331	332	**331**, 295, 267, 217

Rt Retention time, *M* Molecular Weight, Values in bold indicates the base peak ion;a: Compounds Previously reported in *T.baccata*; b: Compounds previously reported in other *Taxus* species;c: Compounds not previously reported in *T. baccata*

base peak at m/z 271 corresponding to apigenin after the neutral loss of one molecule of glucose ($[M+H-162]^+$) (Fig. 3) [35]. The second flavone (Compound (11)) was identified as apigenin, showed $[M+H]^+$ peak at m/z 271, and produced the MS^2 fragments ions at m/z 253 corresponding to the loss of water molecule $[M+H-H2O]^+$ and at m/z 225 corresponding to the loss of a carboxyl group $[M+H-HCOOH]^+$ (Fig. 3) [36]. Both flavones have been identified for the first time in *Taxus baccata* L.

Characterization of flavonol
In this study, nine flavonolswere identified (Table 1). The first flavonol (Compound (12)) was identified as

myricetin 3-*O*-rutinoside, with 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 ($[M+H-308]^+$) [37]. Similarly, two flavonols (compounds 14 and 17), with pseudomolecular ions $[M+H]^+$ of 611 and $[M+H]^+$ of 595, were determined as quercetin 3-*O*-rutinoside and kaempferol 3-*O*-rutinoside, respectively [38, 39].

Three flavonols (Compounds 13, 16 and 19), with molecular formula $C_{15}H_{10}O_8$, $C_{15}H_{10}O_7$ and $C_{15}H_{10}O_6$,also, with pseudomolecular ions $[M+H]^+$ of 319, 303 and 287, were identified as myricetin, quercetin and kaempferol, respectively, after comparing their MS/

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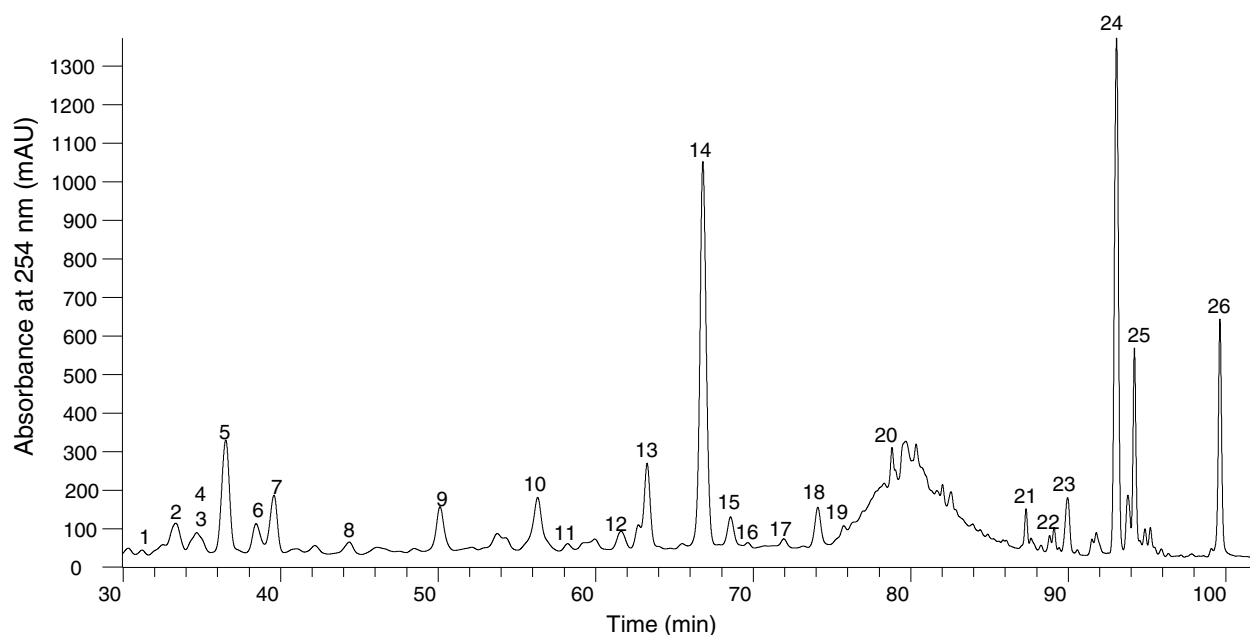
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Fig. 1 HPLC–DAD-MS/MS chromatogram of the methanol extract of Algerian *Taxus baccata* L. 1: (Epi)catechin-(4,8)-(epi)catechin; 2: (Epi) catechin-(4,8)-(epi)gallocatechin; 3: (Epi)gallocatechin-(4,8′)-(epi)gallocatechin; 4: (Epi)gallocatechin; 5: Catechin; 6: unidentified compound; 7: unidentified compound; 8: Epicatechin; 9: Taxifolin; 10: Apigenin 7-*O*-glucoside; 11: Apigenin; 12: Myricetin-3-*O*-rutinoside; 13: Myricetin; 14: Quercetin-3-*O*-rutinoside; 15: Quercetin-7-*O*-glucoside; 16: Quercetin; 17: Kaempferol 3-*O*-rutinoside; 18: kaempferol 7-*O*-glucoside; 19: kaempferol; 20: Isorhamnetin 3-O-rutinoside; 21: Amentoflavone; 22: Robustaflavone; 23: Bilobetin; 24: unidentified compound; 25: Ginkgetin; 26: Sciadopitysin

MS fragmentation patterns with those reported in the literature [40].

Compound (15) was identified as quercetin 7-*O*-glucoside, with pseudomolecular ion $[M+H]^+$ of 467. It produced the MS^2 base peak at m/z 303 corresponding to quercetin after the neutral loss of one molecule of glucose ($[M+H-162]^+$) [38, 41]. Similarly, compound (18), with pseudomolecular ion $[M+H]^+$ of 449 was identified as kaempferol 7-*O*-glucoside [21, 42]. Compound (20) was identified as isorhamnetin 3-*O*-rutinoside, with pseudomolecular ion $[M+H]^+$ of 625. It produced the MS^2 base peak at m/z 317 corresponding to isorhamnetin after the neutral loss of one molecule of rutinose $[M+H-308]^+$(Fig. 3) [41]. This compound has been identified for the first time in *Taxus baccata* L.

Characterization of biflavonoid

Besides, five biflavonoidswere identified (Table 1). The first biflavonoid (Compound 21), with molecular formula C30H18O10 and [M+H]+ at m/z 539, was detected at a retention time of 87.78 min. It produced the MS2 fragments ions at m/z 421, 403 and 377 corresponding to the loss of a 118 Da fragment $[M+H-C8H6O]+$, a 136 Da fragment $[M+H-H2O-C8H6O]+$and a 162 Da fragment $[M+H-C9H6O3]+$, respectively. According to

the above fragmentation, this compound was identified as amentoflavone [42].

The second biflavonoid (Compound 22), with molecular formula $C_{30}H_{18}O_{10}$ and $[M+H]^+$peakat m/z 539, was detected at a retention time of 88.77 min. It generated the MS^2 base peak at m/z 387 $[M+H-C_7H_4O_4]^+$corresponding to the loss of a 152 Da fragment; secondary peaks at m/z 270 $[M+H-C_{15}H_9O_5]^+$ corresponding to the loss of a 269 Da fragment. Based on the above fragmentation, this biflavonoid was assigned as Robustaflavone [42]. This compound has been identified for the first time in *Taxus baccata* L. (Fig. 3).

Three biflavonoid Compounds (23, 25 and 26), with molecular formula $C_{31}H_{20}O_{10}$, $C_{33}H_{24}O_{10}$ and $C_{21}H_{21}O_{11}$, and $[M+H]^+$ at m/z 553, m/z 567 and m/z 581, respectively, were detected at retention times of 90.39, 94.47 and 99.83 min. These compounds were identified as bilobetin, ginkgetin and sciadopitysin, respectively, after comparing their MS/MS fragmentation patterns with those reported in the literature [38, 42].

Unidentified compounds

Two unknown compounds (6 and 7, found only in Algerian *T. baccata* L), eluting at 38.59 and 39.57 min, shared the same pseudomolecular ion at m/z 331 and
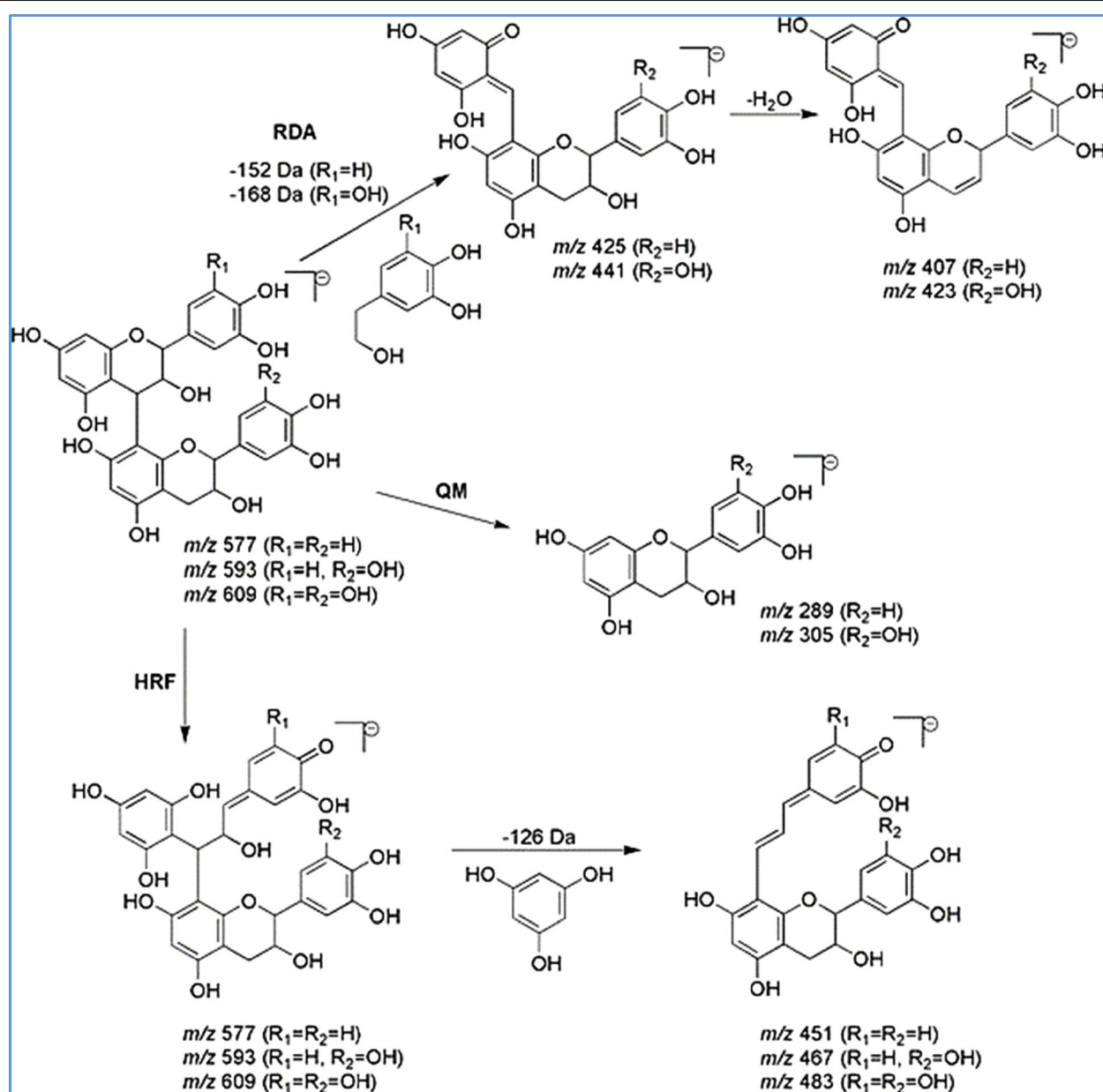
Fig. 2 Fragmentation pathways of dimeric B-type proanthocyanidins (PAs) in *Taxus baccata*. The fragment mechanisms are RDA (retro-Diels–Alder), HRF (heterocyclic ring fission), and QM (quinonemethide)

the same fragmentation pattern, despite different abundances.

Compound 24 showed pseudomolecular ion at m/z 331 in the negative ionization mode. In the MS2 spectrum, it gave a base peak at m/z 331 and secondary ions at m/z 295, 267 and 217, respectively. Its chemical structure remains unknown.

Determination of total flavonoid content

The concentration of the total flavonoid content (TFC) of *Taxus baccata* methanolic extract was determined by the Aluminum Chloride method. The results showed that the *T.baccata* methanolic extract contained a very high amount of flavonoids (Table 2).

Very few studies have been conducted to measure the total flavonoid content of *T. baccata* needles extracts.
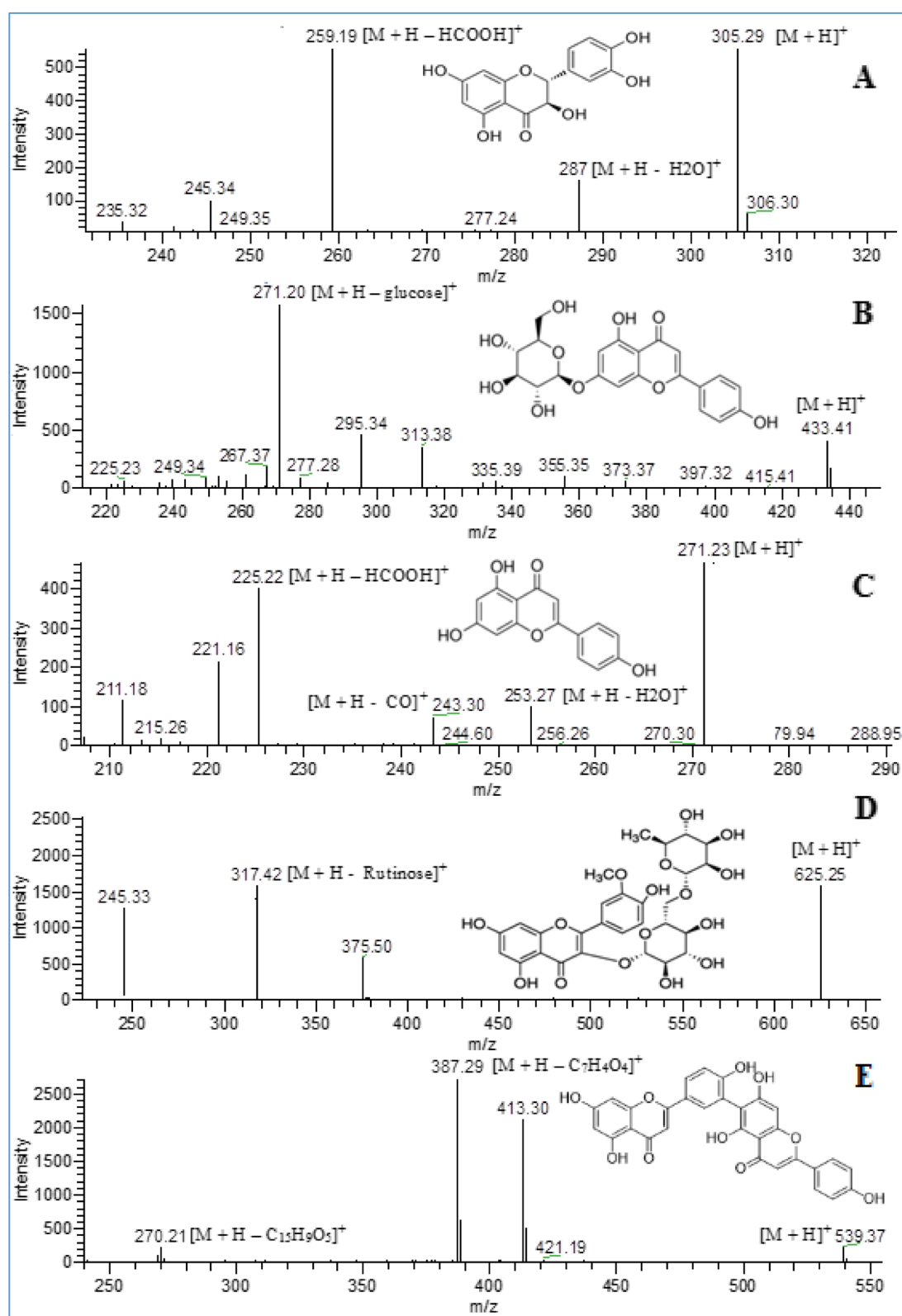
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Fig. 3 MS spectra and chemical structures of new flavonoids detected in *Taxus baccata* by LC– ESI–MS^n. **A**: Taxifolin; **B**: Apigenin 7-O-glucoside; **C**: Apigenin; **D**: Isorhamnetin 3-*O*-rutinoside and **E**: Robustaflavone

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Table 2 Total flavonoid content, radical scavenging and antioxidant activities in the methanolic extract of Algerian *Taxus baccata* L

Extract and standards	TFC (mg RE/g dry extract)	DPPH IC50 (µg/ml)	ABTS IC50 (µg/ml)	FRAP	
				EC50 (µg/ml)	TEAC (µmol TE/g dry extract)
MET	204.26 ± 6.02	35.31 ± 0.29^E	8.27 ± 0.52^D	25.03 ± 0.13^E	377.11 ± 0.29^E
BHT	-	78.96 ± 5.70^F	13.56 ± 0.06^E	22.19 ± 0.5^D	425.05 ± 9.67^D
BHA	-	7.42 ± 0.08^D	1.26 ± 0.10^B	1.61 ± 0.005^C	5677.41 ± 18.54^C
Ascorbic acid	-	3.36 ± 0.14^B	1.27 ± 0.07^B	1.59 ± 0.002^B	5903.56 ± 19.56^B
Trolox	-	4.91 ± 0.16^C	4.03 ± 0.03^C	-	-
Quercetin	-	2.52 ± 0.12^A	1.05 ± 0.02^A	0.98 ± 0.002^A	9572.09 ± 17.11^A

Results are expressed as mean $\pm$ SD. Values with no letters in common are significantly different ($p < 0.05$) for each index. MET: Methanolic extract of *Taxus baccata*; TFC: Total flavonoid content; IC50: The concentration of sample and standard that can inhibit 50% of DPPH or ABTS radicals; EC50: the concentration of sample and standard that can inhibit 50% of FRAP capacity

For example, Milutinović et al. [5], reported that the total flavonoid content of the methanolic extract from the Serbian *Taxus baccata* was 161.98 ± 1.02 mg Rutin equivalent/g dry extract. Moreover, Senol et al. [43], found a total flavonoid content of 48.89 ± 0.76 mg quercetin equivalent/g in the ethanolic extract of the Turkish *Taxus baccata*. Differences in total flavonoid content can be attributed to genetic variation, geographic origins, climatic conditions and plant populations [43, 44]. The results of our investigation are superior to those mentioned above. As a result, it confirms the richness of Algerian *Taxus baccata* L. needle extract in flavonoids.

Free radical scavenging and antioxidant activity

The antioxidant properties of *Taxus baccata* methanolic extract and the positive controls (BHT, BHA, ascorbic acid, trolox and quercetin) have been determined by DPPH, ABTS and FRAP due to their stability, precision and reproducibility [44–46]

The free radical scavenging properties of *Taxus baccata* methanolic extract and the positive controls were presented by their IC_{50} values (Table 2). The IC_{50} is defined as the amount of sample required to scavenge 50% of a given concentration of free radicals. A lower IC_{50} value corresponds with a higher antioxidant property.

The results showed that the antioxidant capacities of the methanolic yew extract [(DPPH $IC_{50} = 35.31 \pm 0.29$ µg/mL and ABTS $IC_{50} = 8.27 \pm 0.52$ µg/mL)] were statistically higher ($P < 0.05$) than the standard substance BHT [(DPPH $IC_{50} = 78.96 \pm 5.70$ µg/mL and ABTS $IC_{50} = 13.56 \pm 0.06$ µg/mL)]. However, all the free radical scavenging activities recorded in the methanolic extract of *Taxus baccata* were significantly ($P < 0.05$) lower than those BHA, ascorbic acid, trolox and quercetin (Table 2).

On the other hand, the methanolic extract of *T. baccata* and all the positive controls showed the ferric reducing ability when assayed with FRAP reagent, however, their capacities were observed differently. Quercetin showed the highest (EC50 = 0.98 ± 0.002 µg/mL; TEAC = 9572.09 µmol TE/g dry extract) while the methanolic extract of *T. baccata* showed the lowest ferric reducing ability (EC50 = 25.03 ± 0.13 µg/mL; TEAC = 377.11 ± 0.29 µmol TE/g dry extract). The ranking order for reducing power was quercetin > ascorbic acid > BHA > BHT > methanolic extract (Table 2).

Our results agree with those of Guleria et al. [47] and Milutinović et al. [5] which mentioned that the *Taxus baccata* methanolic extracts have strong antioxidant properties, acting as free radical scavengers and metal ion reducing agents.

From literature, the FRAP, DPPH and ABTS are reactive towards most antioxidants including flavonoid compounds [48–50]. Many flavonoids are found to be strong antioxidants effectively scavenging the DPPH and ABTS radicals because of their hydroxyl groups [49–51]. For instance, quercetin, catechin, and epicatechin have five hydroxyl groups attached to them which make them potent antioxidant and radical scavengers [52]. Taxifolin present in several plants, including *T. baccata*, has been reported to scavenge DPPH and ABTS free radicals [53, 54]. Other flavonoids like proanthocyanidins, amentoflavone, kaempferol, myricetin, isorhamnetin and apigenin have been reported to scavenge free radicals [55–58].

In the FRAP assay, the presence of the reductants in the solution causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by absorbance measurement at 539 nm [59]. Previous studies revealed that the reducing properties have been shown to exert antioxidant action by donating a hydrogen atom to break the free radical chain. Increasing absorbance at 539 nm indicates an increase in reducing ability [49, 59]. The antioxidants

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present in the methanolic extract of *T. baccata* caused their reduction of Fe^{3+}/ ferricyanide complex to the ferrous form, and thus proved the reducing power.

Our study revealed the presence of twenty-three flavonoids in the methanolic extract of Algerian *T. baccata* which play an important role in the ferric reducing ability FRAP and in neutralizing DPPH and ABTS radicals.

Conclusion

In conclusion, our research investigated for the first time the flavonoid compound profile from extracts of *Taxus baccata* L. needles growing in Algeria and evaluated their antioxidant activities. The analysis of the methanolic extract by HPLC–DAD–ESI–MS/MS showed the presence of 23 flavonoid compounds including 6 flavon-3-ols, 1 flavanonol, 2 flavones, 9 flavanols and 5 biflavonoids. These bioactive compounds have very valuable antioxidant properties, acting as free radical scavengers and metal ion reducing agents confirming the medicinal interest of this plant. Our results reveal that the potential of *Taxus Baccata* L. as a source of natural bioactive molecules can be exploited in the food and pharmaceutical field.

Declaration

Abbreviations
ABTS: 2,2'-Azino-bis3-ethylbenzothiazoline-6-sulphonic acid; DPPH: 2,2-Diphenyl-1picrylhydrazyl; FRAP: Ferric Reducing Antioxidant Power Assay; HPLC–DAD–ESI–MS/MS: High performance liquid chromatography with diode-array detection coupled to electrospray ionisation tandem mass spectrometry; EC50: The concentration of sample and standard that can inhibit 50% of FRAP capacity; IC_{50}: The concentration of sample and standard that can inhibit 50% of DPPH and ABTS radicals; TFC: Total flavonoid content.

Authors' contributions
MB collected the samples, performed all the experiments. CP performed the HPLC–DAD-ESI–MS/MS analysis. LK, AM and M.K-S supervised the study design. MB drafted the manuscript with AM, RB and SK. All authors read the manuscript and approved the final version.

Availability of data and materials
All data generated or analysed during this study are included in this article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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