IJPSR (2021), Volume 12, Issue 5 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 23 April 2020; received in revised form, 09 July 2020; accepted, 23 August 2020; published 01 May 2021

FREE RADICAL SCAVENGING ACTIVITY AND DETAILED FLAVONOID PROFILING OF ALGERIAN YEW (*TAXUS BACCATA* L.) BY LC–ESI–MS/MS

M. Bekhouche *, R. Benyammi, M. Khelifi Slaoui, L. Khelifi and A. Morsli

Department of Plant Production, Higher National Agronomic School, 16200, El Harrach-Algiers, Algeria.

Keywords:

Algerian yew, Antioxidant property, Flavonoids, Free radicals, LC–MS

Correspondence to Author:
M. Mohamed Bekhouche

Department of Plant Production, Higher National Agronomic School, Hassan Badi, 16200, El Harrach-Algiers, Algeria.

E-mail: mohamed.bekhouche@edu.ensa.dz

ABSTRACT: According to recent studies, the antioxidant related properties of *Taxus baccata* L. needle extracts are remarkable making it a possible raw material for the pharmaceutical industry, hence requiring the identification of major bioactive compounds and the assessment of the antioxidant properties of the extracts. The aim of this study was to investigate for the first time the individual flavonoids and the in vitro free radical scavenging activity of the methanol needle extracts from two Algerian *Taxus baccata* populations. The identification process was carried out using liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC–ESI–MS/MS). A total of twenty compounds have been characterized and identified. Some of which have never been previously reported in *T. baccata* L. The total flavonoid contents in *Taxus baccata* showed significant differences ($p<0.05$) according to the populations. The total flavonoid contents were relatively greater at the Chrea population (TFC = 220.1 ± 6.36 mg RE/g dry extract) compared to the Tikjda population (TFC = 166.6 ± 1.73 mg RE/g dry extract). Similarly, the highest antioxidant activity was observed with the methanolic extracts from the Chrea population [(DPPH IC_{50} = 29.44 ± 0.99 µg/ml, ABTS IC_{50} = 10.94 ± 1.06 µg/m). The findings indicated that the *Taxus baccata* needles possessed strong antioxidant properties and could be an important source of natural compounds for development of new drugs.

INTRODUCTION: Free radicals are formed continuously in the cells as part of normal cellular function; nevertheless, excess production might play a role in the pathophysiology of many disease conditions, including cardiovascular diseases, Alzheimer's disease, diabetes, and cancer [1, 2]. The protection against oxidative damage and chronic diseases is achieved through antioxidants [3]. In recent years, there has been an increasing interest in the discovery of natural antioxidants as they can protect the human body from free radicals and slow the progression of some chronic diseases [4, 5].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(5).2613-19

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(5).2613-19

Natural antioxidants are widely distributed in medicinal plants. These natural antioxidants, especially flavonoids, play an important role in the prevention of degenerative diseases, particularly cancers, neurodegenerative diseases and cardio-vascular diseases [6, 7].

The genus *Taxus*, commonly named as yew, is a gymnosperm in the family *Taxaceae*which includes nine species of small trees or shrubs [8]. One of the species, *Taxus baccata*, is represented in Algeria. In folk medicine, needles of yew are used to treat asthma, bronchitis, epilepsy and diarrhea [9]. Moreover, previous studies reported that *Taxus baccata* L. contains a diversity of bioactive compounds including phenolic compounds and flavonoids [10, 11] *etc*., showing many biological benefits as anticancer, anti-mutagenic, anti-inflammatory and anti-HIV properties [12, 13].

Despite the fact that flavonoid compounds were previously reported as constituents of the *Taxaceae* family plants [14, 15], only two studies have been carried out on the flavonoid composition of the yew tree [11, 16], and little is known about the flavonoids of the plant's needles. This is the first study of the identification of flavonoids of Algerian *Taxus baccata* L. Therefore, the present research aims to characterize the flavonoid compounds of methanol extract from two Algerian *Taxus baccata* populations using liquid chromatography coupled with mass spectrometry (LC–ESI–MS/MS). Another objective was to measure the total phenolic content (TPC) and total flavonoid content (TFC) and further analyze their antioxidant capacity using DPPH and ABTS radical-scavenging activity.

MATERIALS AND METHODS:

Plant Collection: 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 BENHOUHOU, Department of Plant Sciences, Algerian Higher National Agronomic School. Samples were, then, shade dried at room temperature and ground into fine powder.

Preparation of Flavonoid Sample: The extraction of flavonoid compounds is carried according to the protocol of Oosthuizen [17] with slight modifications. 20 grams of needle powder were extracted with 200 mL methanol during 6 h in a conventional Soxhlet apparatus. Subsequently, the filtrate was concentrated to dryness in a rotary evaporator (Büchi R II V-700). The obtained crude methanol extracts were stored in dark glass bottles at 4 °C until used for further analyses.

LC–ESI–MS/MS Determination of Flavonoids: The flavonoids present in the methanolic extracts of *T. baccata* were identified using an LC–ESI–MS/MS. 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). Five microliters of the methanolic extracts were separated on a C18 Alltima (150mm × 2.1mm) column (Grace/Alltech, Darmstadt, Germany). The flow rate was set at 200 µl/min, and mobile phases consisted in water modified with formic acid (0.1%) for A and acetonitrile modified with formic acid (0.1%) for B. 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+). 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 in order to identify the nature of the metabolites.

Total Phenolic Content (TPC): Total phenol content was measured using the Folin–Ciocalteu method[18] applying gallic acid as the standard at 765 nm. Total phenol content was expressed in milligrams gallic acid equivalent per gram of dry extract (mg GAE/g dry extract).

Total Flavonoid Content (TFC): The total flavonoid assay was run as described by Zhisen [19] using rutin as the standard at 510 nm. The total flavonoid content was expressed in milligrams rutin equivalents per gram of dry extract (mg R.E/g dry extract).

Free Radical Scavenging Activity Analysis:

DPPH Radical-Scavenging Activity Assay: The DPPH radical scavenging activity of the methanol extracts of *T. baccata* was measured as described byPatra [20] with modifications. The test was performed on a 96-well microplate mixing 80 µl of the methanolic extract at different concentrations or positive control (BHT) 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 DPPH scavenging capacity of the extract was determined using the following formula:

DPPH radical scavenging activity (%) = $\{(A_C - A_S)/ A_C\} \times 100$

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

ABTS Free Radical-Scavenging Activity Assay: The ABTS free radical scavenging ability of the methanol extracts of *T. baccata* was determined according to the method described byLe Grandois [21] with modifications. Briefly, in a 96-well microplate, 80 µl volume of the methanolic extract at different concentrations or positive control (BHA) 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. The test was repeated three times for each concentration. The ABTS scavenging capacity of the extract was determined using the following formula:

ABTS radical scavenging activity (%) = $[(A_C – A_S)/A_C] \times 100$

Where A_C = absorbance of the control and A_S = absorbance of the test extracts.

RESULTS AND DISCUSSION:

LC–ESI–MS/MS Analysis of Flavonoid Compounds: The flavonoid 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–ESI–MS/MS) in positive mode. The methanolic yew extracts showed similar flavonoid profiles. The identified flavonoids are listed in **Table 1**, where the compounds are numbered according to their retention times in the obtained chromatograms **Fig. 1**.

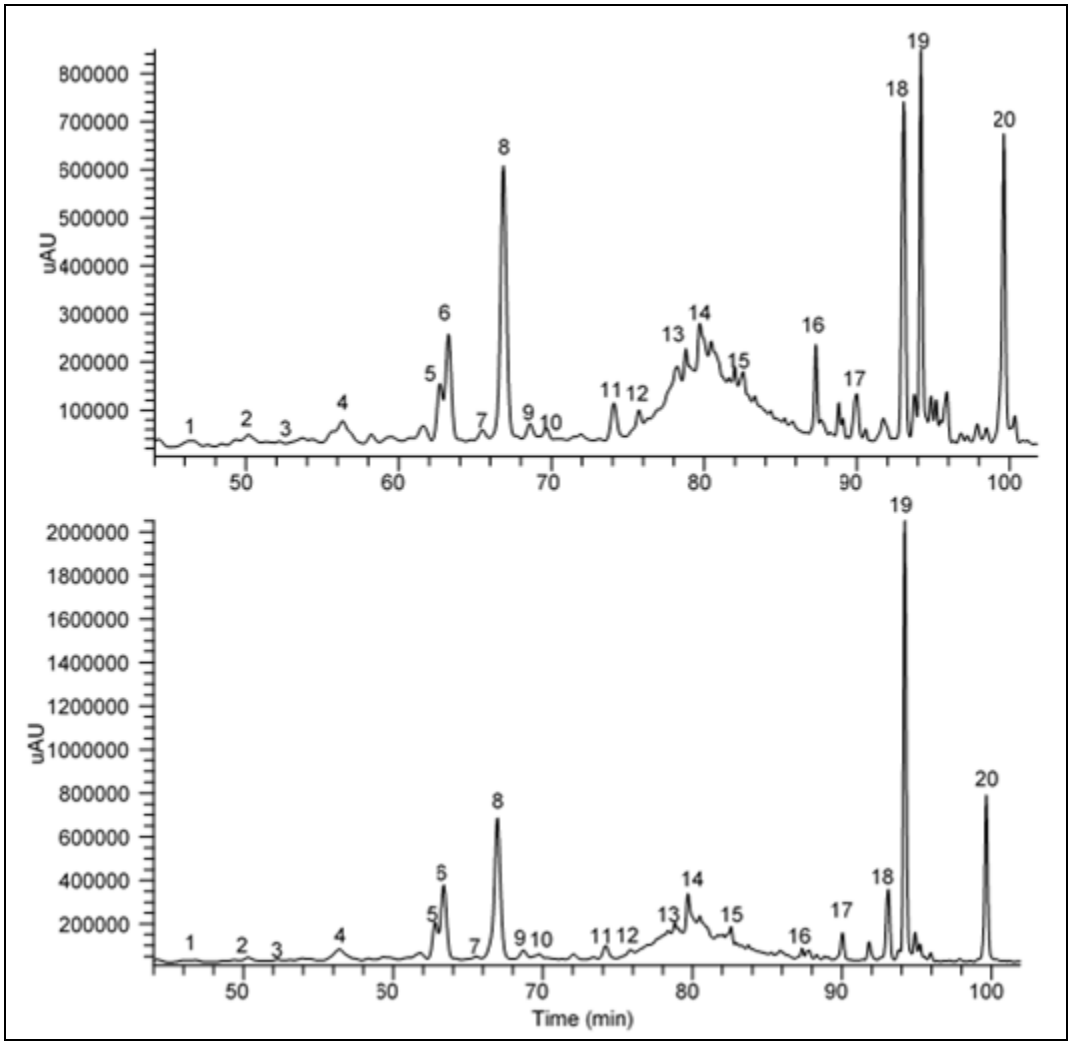

FIG. 1: HPLC- CHROMATOGRAM OF THE METHANOL EXTRACTS OF ALGERIAN *TAXUS BACCATA* L. A): CHREA POPULATIONAND B): TIKJDA POPULATION

A total of twenty compounds were characterized and identified based on their LC retention times, mass data generated by LTQ-MS, MS/MS fragmentation patterns, or by comparison with literature data.

The first flavonoid (compound (1)) was identified as taxifolin, with pseudomolecular ion $[M+H]^+$ of 305. It produced the MS^2 fragments ions at m/z 287 corresponding to the loss of water molecule $[M + H - H_2O]^+$ and at m/z 259 corresponding to the loss of a carboxyl group $[M + H - HCOOH]^+$ **Fig. 2**. The second flavonoid (Compound (2)) 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 $[M + H - H_2O]^+$ and at m/z 225 corresponding to the loss of a carboxyl group $[M + H - HCOOH]^+$ **Fig. 2**. The third flavonoid (compound (3)) was identified as isorhamnetin, with pseudomolecular ion $[M+H]^+$ of 317. It generated the MS^2 fragments ions at m/z 299 corresponding to the loss of water molecule $[M + H - H2O]^+$ and at m/z 271 corresponding to the loss of a carboxyl group $[M + H - HCOOH]^+$ **Fig. 2**. These flavonoids have been detected for the first time in *T. baccata*.

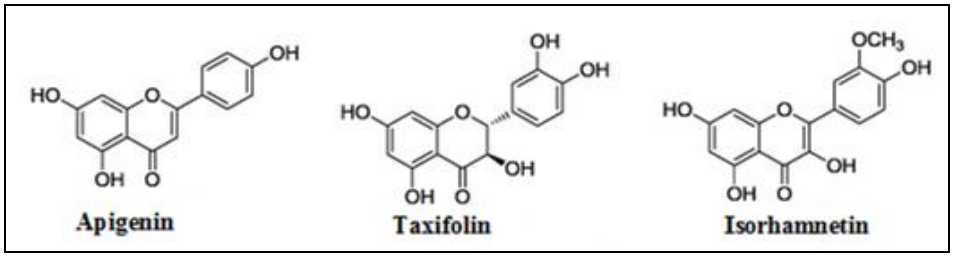

FIG. 2: CHEMICAL STRUCTURES OF NEW FLAVONOIDS DETECTED IN ALGERIAN *TAXUS BACCATA* L. BY LC– ESI–MS/MS

Compound (4) showed pseudomolecular ion at m/z 373 in the negative ionization mode. In the MS^2 spectrum, it gave a base peak at m/z 313 and secondary ions at m/z 295 and 255, respectively. Its chemical structure remains unknown. Compound (5) with a retention time of 62.91 min and $[M + H]^+$ at m/z 319 produced the MS^2 fragments ions at m/z 301 $[M + H - 16]^+$ and at m/z 273 $[M + H - HCOOH]^+$. Compound (6) was identified as myricetin *O*–hexoside, with pseudomolecular ion $[M+H]^+$ of 481. It produced the MS^2 base peak at m/z 319 corresponding to myricetin after the neutral loss of one molecule of hexose $[M+H - 162]^+$.

Compound (7) 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, compounds 12 and 13 with t_R 75.49 and 79.01 min were assigned as kaempferol 7-*O*-glucoside and apigenin 7-*O*- glucoside [11].

Compound (8) (t_R 59.21 min) was identified as quercetin 3-*O*-rutinoside with a pseudomolecular ion $[M+H]^+$of 611. It produced the MS^2 base peak at m/z 303 corresponding to quercetin after the neutral loss of one molecule of rutinose ($[M+H - 308]^+$). Similarly, compound 11 with t_R 74.10 min was assigned as kaempferol 3-*O*-rutinoside [11].

Compounds 9 and 10 (t_R 68.90 and 69.86 min) with pseudomolecular ions $[M+H]^+$ of 303 and 287 were assigned as quercetin and kaempferol, respectively, after comparing their retention times and MS/MS fragmentation patterns with those reported in literature [16].

Two unknown compounds (14 and 15, found only in Algerian *T. baccata* L), eluting at 79.91 and 82.82 min, shared the same MS^2 base peak at m/z 194, despite different fragmentation patterns.

Compounds 16, 17, 19 and 20 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 539, at m/z 553, at m/z 567 and at m/z 581 were detected at retention times of 87.52, 90.21, 94.40 and 99.83 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 [11].

Compound 18 showed pseudomolecular ion at m/z 331 in the negative ionization mode. In the MS^2 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.

TABLE 1: IDENTIFICATION OF FLAVONOID COMPOUNDS IN *TAXUS BACCATA* L. BY LC-ESI-MS/MS IN POSITIVE MODE

S. no.	Rt (min)	MW	$[M+H]^+$ (m/z)	HPLC-ESI-MS^n (m/z)	Molecular Formula	Identification
1	46.16	304	305	305, 287, 259, 231	$C_{15}H_{12}O_7$	Taxifolin,[b]
2	48.01	270	271	271, 243, 225	$C_{21}H_{20}O_{10}$	Apigenin [c]
3	53.34	316	317	317, 299, 271	$C_{16}H_{12}O_7$	Isorhamnetin [c]
4	56.52	372	373	313, 295, 255	-	unidentified
5	62.91	318	319	319, 301, 273	$C_{15}H_{10}O_8$	Myricetin[a,b]
6	63.47	480	481	481, 319	$C_{21}H_{20}O_{13}$	Myricetin *O*-hexoside [c]
7	65.67	464	465	303, 255, 151	$C_{21}H_{20}O_{12}$	Quercetin-7-*O*-glucoside [a,b]
8	67.06	610	611	303	$C_{27}H_{30}O_{16}$	Quercetin-3-*O*-rutinoside[a,b]
9	68.80	302	303	303, 153, 137, 121	$C_{15}H_{10}O_7$	Quercetin [a,b]
10	69.86	286	287	287, 269, 241, 213, 153	$C_{15}H_{10}O_6$	kaempferol [a,b]
11	74.10	594	595	287	$C_{27}H_{30}O_{15}$	Kaempferol-3-*O*-rutinoside [a,b]
12	75.49	448	449	287	$C_{21}H_{20}O_{11}$	kaempferol 7-*O*-glucoside [a,b]
13	79.01	432	433	271	$C_{21}H_{20}O_{10}$	Apigenin 7-O-glucoside [a]
14	79.91	584	584	194, 313, 253	-	unidentified
15	82.82	583	584	407, 313, 194	-	unidentified
16	87.52	538	539	539, 403, 421, 377	$C_{30}H_{18}O_{10}$	Amentoflavone [a,b]
17	90.21	552	553	553, 435, 391, 297	$C_{31}H_{20}O_{10}$	Bilobetin[a]
18	93.24	330	331	331, 295, 217	-	unidentified
19	94.40	566	567	567,449, 417, 405	$C_{32}H_{20}O_{10}$	Ginkgetin [a]
20	99.83	580	581	581, 549, 449, 415	$C_{33}H_{24}O_{10}$	Sciadopitysin [a,b]

Rt, retention time; MW: Molecular Weight; Values in bold indicate 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*

Determination of Total Flavonoid Content (TFC): 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 **Fig. 3**.

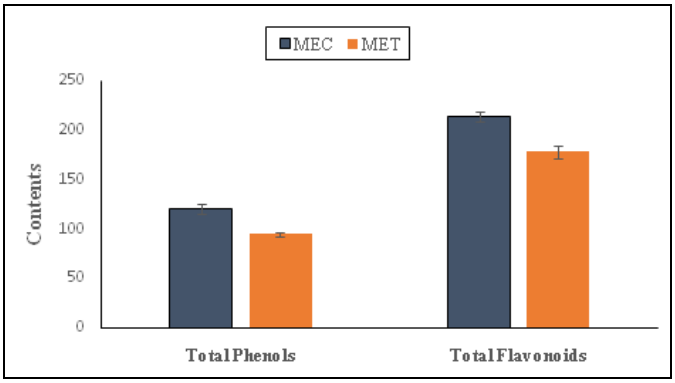

FIG. 3: TOTAL PHENOL (GAE/g dry EXRACT) AND FLAVONOID (RE/g dry EXTRACT) CONTENTS IN THE METHANOLIC EXTRACTS FROM TWO ALGERIAN *T. BACCATA* POPULATIONS. Values are expressed as mean ± standard deviation of triplicate. Statistical significance 1% level ($p \leq 0.05$).MEC: Methanolic extracts from Chrea; MET: Methanolic extract from Tikjda

The results showed that the *T. baccata* methanolic extracts were found to contain very high amounts of phenols and flavonoids **Fig. 2**. The total phenolic and flavonoid contents were significantly ($p<0.05$) greater in the methanolic extract from Chrea (TPC= 120.57 ± 5.25 mg GAE/g dry extract; TFC = 214.1 ± 4.94 mg RE/g dry extract) compared to the methanolic extracts from Tikjda (TPC= 94.5 ± 2.09 mg GAE/g dry extract; TFC = 178.1 ± 6.36 mg RE/g dry extract). Probably, the differences in total phenolic and flavonoid contents could be attributed to genetic variation, distinct environmental, geographic origins, climatic conditions and plant populations [23].

Very few studies have been conducted to measure the total phenol and flavonoid content of *T. baccata* needles extracts. For example, Guleria [24] found a total phenol content of 69.96 ± 2.73 mg GAE/g in the methanol extract of the Indian *Taxus baccata*.Milutinović et al., [25] reported that the total phenolic and 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 provenance of Chrea.

Free radical scavenging activity Analysis: The antioxidant and the radical scavenging capacities of the methanolic extracts of the two *Taxus baccata* populations and the positive control (BHT) have been determined by the two well-known methods (DPPH and ABTS) due to their stability, reproducibility and precision [26, 27].

Statistical analysis indicated that there was a significant difference ($P<0.05$) between the two populations for their antioxidant activity. The highest antioxidant activities were obtained from the methanolic extracts from the Chrea population (DPPH = 87.63 ± 2.22 % and ABTS = 90.16 ± 0.52 %). The antioxidant capacities of the same extract were statistically higher than the synthetic antioxidant BHT (DPPH = 71.75± 9.54 µg/ml, ABTS = 42.63 ± 2.23 %) **Fig. 4**.

From literature, the DPPH and ABTS are reactive towards most antioxidants including flavonoid compounds [28]. Many phenolic compounds such as flavonoids are found to be strong antioxidants effectively scavenging the DPPH and ABTS radicals because of their phenolics hydroxyl groups [28, 29]. Our study revealed the presence of twenty bioactive compounds in the methanolic extracts of Algerian *T. baccata*, which might play an important role inabsorbing and neutralizing free radicals. In this context, the results of our investigation are in accordance with the studies published by Mukherjee [30] and Milutinović [25], which mentioned that the *Taxus baccata* needles extracts have strong antioxidant properties, acting as DPPH and ABTS free radical scavengers.

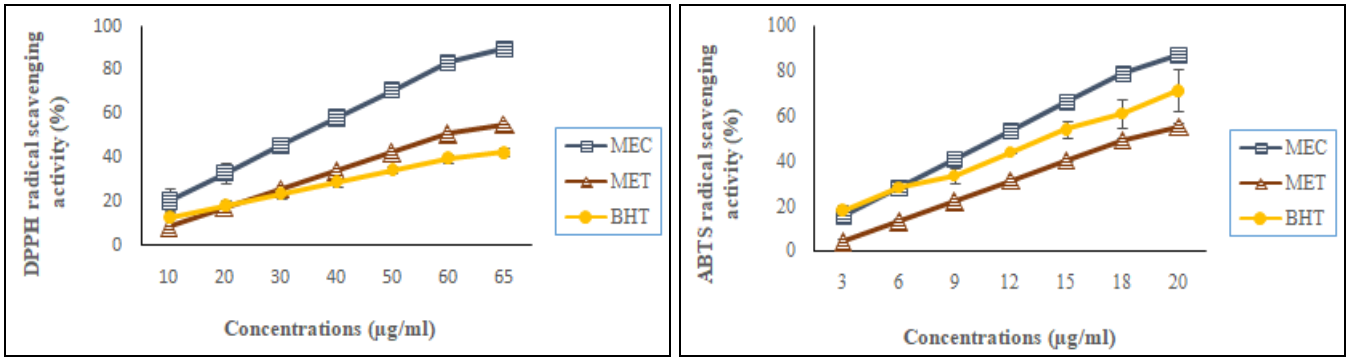

FIG. 4: RADICAL SCAVENGING ACTIVITY FROM THE METHANOL EXTRACTS OF ALGERIAN *T. BACCATA* USING DPPH AND ABTS ASSAYS. Values are expressed as mean ± standard deviation of triplicate. Statistical significance 1% level ($p\leq0.05$).MEC: Methanolic extracts from Chrea; MET: Methanolic extract from Tikjda

CONCLUSION: In this study, we investigated for the first time the flavonoid compound profile and evaluated total phenolic and flavonoid contents in addition to antioxidant activities of needles extracts obtained from two *Taxus baccata* L. populations growing in Algeria. Our findings revealed that the methanolic extracts were found to have very high flavonoid contents. The analysis of the methanol extracts by LC–ESI–MS/MS showed the presence of twenty bioactive compounds confirming the medicinal interest of this plant. These compounds have very valuable antioxidant properties, acting as free radical scavengers. 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.

ACKNOWLEDGEMENT: The authors wish to thank Professor Professor BENHOUHOU (Department of Plant Sciences, Algerian Higher National Agronomic School) for the identification of the plant.

CONFLICTS OF İNTEREST: The authors declared no conflict of interest.

REFERENCES:

1. Bhatti J, Bhatti G and Reddy P: Mitochondrial dysfunction and oxidative stress in metabolic disorders—A step towards mitochondria-based therapeutic strategies. Biochimica et Biophysica Acta 2017; 1863: 1066-77.
2. Di Meo S and Venditti P: Evolution of the knowledge of free radicals and other oxidants. Oxidative Medicine and Cellular Longevity 2020; 2020: 1-32.
3. Sharifi-Rad M, Anil Kumar N and Zucca P: Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the

Pathophysiology of Chronic Diseases. Front Physiol 2020; 11: 494.
4. Rahman M, Hossain A and Mostofa M: Evaluation of anti-ROS and anticancer properties of *Tabebuia pallida* L. Leaves. Clin Phytosci 2019; 5: 17.
5. Tan BL, Norhaizan M, Liew W and Sulaiman Rahman H: Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. Front Pharmacol 2018; 9: 1162.
6. Ganesan K and Xu B: Polyphenol-Rich Lentils and Their Health Promoting Effects. Int J Mol Sci 2017; 18: 2390.
7. Ullah A, Munir S and Badshah S: Important Flavonoids and Their Role as a Therapeutic Agent. Molecules 2020; 25: 5243.
8. Coughlan P, Carolan J, Hook I, Kilmartin L and Hodkinson T: Phylogenetics of Taxus using the internal transcribed spacers of nuclear ribosomal DNA and Plastid trnL-F Regions. Horticulturae 2020; 6: 19-36.
9. Juyal D, Thawani V, Thaledi S and Joshi M: Ethnomedical Properties of *Taxus wallichiana* Zucc. (Himalayan Yew). J Tradit Complement Med 2014; 4: 159-61.
10. Feucht W, Schmid M and Treutter D: DNA and Flavonoids leach out from active nuclei of taxus and tsuga after extreme climate stresses. Plants 2015; 4: 710-27.
11. Krauze-Baranowska M: Flavonoids from the genus Taxus. Z Naturforsch, C, J Biosci 2004; 59: 43-47.
12. Chahar MK, Sharma N, Dobhal MP and Joshi Y: Flavonoids: A versatile source of anticancer drugs. Pharmacogn Rev 2011; 5: 1-12.
13. Nisar M, Khan I, Ahmad B, Ali I, Ahmad W and Choudhary M: Antifungal and antibacterial activities of *Taxus wallichiana* Zucc. Journal of Enzyme Inhibition and Medicinal Chemistry 2008; 23: 256-60.
14. Wang YF, Yu SH, Dong M, Zhang ML, Huo CH and Shi QW: Chemical Studies on *Taxus cuspidata*. Chem Biodivers 2010; 7: 1698-16.
15. Li C, Huo C, Zhang M and Shi Q: Chemistry of Chinese yew, *Taxus chinensisvar*. mairei. Biochemical Systematics and Ecology 2008; 36: 266-82.
16. Vignolini P, Gehrmann B, Melzig M, Borsacchi L, Scardigli A and Romani A: Quality control and analytical test method for *Taxus baccata* tincture preparation. Nat Prod Commun 2012; 7: 875-77.
17. Oosthuizen D, Goosen NJ and Stander MA: Solvent Extraction of Polyphenolics from the Indigenous African Fruit *Ximenia caffra* and Characterization by LC-HRMS. Antioxidants2018; 7: 103.
18. Singleton VL, Orthofer R and Lamuela-Raventós RM: Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In: Methods in Enzymology. Vol 299. Elsevier; 1999: 152-78.
19. Zhishen J, Mengcheng T and Jianming W: The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 1999; 64: 555-59.
20. Patra JK, Kim SH and Baek K-H: Antioxidant and free radical-scavenging potential of essential oil from *Enteromorpha linza* L. prepared by microwave-assisted hydrodistillation. Journal of Food Biochemistry 2015; 39: 80-90.
21. Le Grandois J, Guffond D, Hamon E, Marchioni E and Werner D: Combined microplate-ABTS and HPLC-ABTS analysis of tomato and pepper extracts reveals synergetic and antagonist effects of their lipophilic antioxidative components. Food Chemistry 2017; 223: 62-71.
22. Lin L-Z and Harnly JM: A Screening Method for the Identification of Glycosylated Flavonoids and Other Phenolic Compounds Using a Standard Analytical Approach for All Plant Materials. J Agric Food Chem 2007; 55: 1084-96.
23. Bajalan I, Mohammadi M, Alaei M and Pirbalouti AG: Total phenolic and flavonoid contents and antioxidant activity of extracts from different populations of lavandin. Industrial Crops and Products 2016; 87: 255-60.
24. Guleria S, Tiku AK, Singh G, Koul A, Gupta S and Rana S: In vitro antioxidant activity and phenolic contents in methanol extracts from medicinal plants. J Plant Biochem Biotechnol 2013; 22: 9-15.
25. Milutinović M, Stanković M, Cvetković D, Topuzović M, Mihailović V and Marković S: Antioxidant and anticancer properties of leaves and seed cones from European yew (*Taxus baccata* L.). Archives of Biological Sciences 2015; 67: 525-34.
26. Becker M, Nunes G and Ribeiro D: Determination of the Antioxidant Capacity of Red Fruits by Miniaturized Spectrophotometry Assays. Journal of the Brazilian Chemical Society 2019; 30: 1108-14.
27. Hernández-Rodríguez P, Baquero LP and Larrota HR. Chapter 14 - Flavonoids: Potential Therapeutic Agents by Their Antioxidant Capacity. In: Campos MRS, ed. Bioactive Compounds. Woodhead Publishing 2019: 265-88.
28. Apak R, Güçlü K and Demirata B: Comparative Evaluation of Various Total Antioxidant Capacity Assays Applied to Phenolic Compounds with the CUPRAC Assay. Molecules 2007; 12: 1496-1547.
29. Khan RA, Khan MR, Sahreen S and Ahmed M: Assessment of flavonoids contents and *in-vitro* antioxidant activity of *Launaea procumbens*. Chem Cent J 2012; 6: 43-44.
30. Mukherjee A, Joshi K, Pal RS, Atheequlla GA, Roy ML and Chandra N: Scientific health benefits of Namakeen Chai/Jya (salted tea): A traditional tea beverage of Bhotiya tribal community in higher altitudes of Uttarakhand. Indian Journal of Traditional Knowledge 2018; 17: 365-69.

How to cite this article:
Bekhouche M, Benyammi R, Slaoui MK, Khelifi L and Morsli A: Free radical scavenging activity and detailed flavonoid profiling of Algerian yew (*Taxus baccata* L.) by LC–ESI–MS/MS. Int J Pharm Sci & Res 2021; 12(5): 2613-19. doi: 10.13040/IJPSR.0975-8232.12(5).2613-19.