

Phytochemical analysis and antioxidant activity of twigs and leaves extracts of *Tertraclinis articulata* (Vahl) Masters

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Abstract

To research natural antioxidants, the present study aims to extract and identify new substances of natural origin through the phytochemical study and the evaluation of the antioxidant activity of extracts of twigs and leaves of *Tetraclinis articulata* (Vahl) Masters, harvested from the Khenifra region (Middle Atlas, Morocco). The Phytochemical screening, based on precipitation and complexation reactions, showed that the twigs and leaves of *T. articulata* contain flavonoids, tannins, alkaloids, mucilages, sterols and triterpenes. The extraction of the phenolic compounds was done using a Soxhlet apparatus. The assays of polyphenols, flavonoids and condensed tannins of the obtained extracts showed that the highest content of polyphenols was recorded by the ethyl acetate extract ($654.69 \pm 0.33 \text{mgEAG/gE}$). Concerning the flavonoids, the hydroethanolic extract records the highest content ($20,31 \pm 0,02 \text{mgEQ/gE}$). For the condensed tannins, the highest content was observed by the extract of the residual phase ($27.72 \pm 0.11 \text{mgEC/gE}$). Indeed, due to the analysis by HPLC/SM, we were able to identify in the E.MeOH of *T. articulata* seven flavonic glycosides which are Catechin, B-type (Epi) catechin dimer, gallocatechin, Myricetin-hexose, Myricetin-rhamnose, Quercetin-3-o-Rhamnoside and kampferol-deoxyhexose. The in vitro evaluation of the antioxidant activity of *T. articulata* extracts was performed by DPPH and FRAP methods. The extracts of *T. Articulata* showed a significant antioxidant power. The lowest IC_{50} and $\text{EC}_{0.5}$ were recorded by E. EtOH. These low concentrations (IC_{50} and $\text{CE}_{0.5}$) obtained by DPPH and FRAP are $70 \pm 0.07 \mu\text{g/ml}$ and $104.12 \pm 1.02 \mu\text{g/ml}$, respectively. This study revealed that the extracts of *T. articulata* are potentially important due to their richness in secondary metabolites of its antioxidant power.

1. Introduction

Since its existence, Man has depends on plants to relieve his ailments and to find the energy he needed through the consumption of fruits, vegetables, etc. Although medicines, as we know them today, were not available in the early days of human existence. through the consumption of plants (fruit, leaves, roots, etc.) our ancestors were able to find ways to heal. Over time, they have accumulated experience that allowed them to learn about the medicinal properties of plants. Even today, despite the development and availability of modern medicines, people all over the world still use this knowledge to restore their health. Even better, this knowledge is in some cases the basis on which more than 20% of the known medicines have been developed [1]. In Morocco, the use of plants for health has always occupied a prominent place in the lives of the people. In fact, besides the use of synthetic medicines, in each region, there is a family self-treatment inspired by the traditional heritage as well as the use of traditional healers [2]. The latter, which are few and composed of elderly and mostly illiterate people, are the repositories of this knowledge [3]. *Tetraclinis articulata* (Vahl) has been used since ancient times in traditional medicine, and in the 17th century, it was called the "tree of life" due to its various medicinal properties attributed to the balsamic resin. The extract of this resin increases blood pressure and lowers the fever. Several studies indicate that the local Moroccan population uses this plant in traditional medicine. More specifically, the people of the Khenifra region use this essence of the forest to treat many diseases. For example, the botanical and ethno-study of medicinal plants used in Khenifra conducted by Hachi et al., 2015 [4], showed that a

decoction of the leaves of *T. articulata* (Vahl) is prescribed to treat gastrointestinal pain and diabetes. In addition, a pharmacological ethnographic study conducted in eastern Morocco shows that the leaves of Thuja Berberia are used against diabetes and hypertension [5]. The different parts of the tree, especially the leaves and twigs, are used in the treatment of infections of the intestines and respiratory system [2]. Thus, a decoction of the leaves is indicated for the treatment of gastrointestinal pain. Thuya leaf powder combined with henna (*Lawsonia inermis* L.) is applied to the scalp as a softener and anti-hair loss. The decoction of leaves and fruits in low doses is indicated against stomach diseases, acidity, diuretics, menstruation, antirheumatics, liver diseases and atherosclerosis [6]. The present work aims to contribute to the valorization of this species through chemical characterization and evaluation of the antioxidant activity of extracts of twigs and leaves of *Tetraclinis articulata* (Vahl) harvested from the Khenifra region (Morocco).

1. Material And Methods

2.1. Plant material

The leafy twigs of *T. articulate* Vahl (Fig. 1) were harvested in the rural commune of El hammam (Khenifra-Middle-Atlas Morocco), then they were dried in the shade for 10 days. The botanical identification of the species was carried out in the Floristics laboratory at the Scientific Institute in Rabat.

2.2. Phytochemical screening

Phytochemical screening is a qualitative study based on coloring and precipitation reactions, by specific chemical reagents, carried out on extracts of *T. articulata*. These characterization tests of different chemical groups were performed according to the protocol of [7] [8] [9].

2.3. Extraction and fractionation of polyphenols from *T. articulata*

a soxhlet-type extractor was used to extract the polyphenols, $m_1 = 30$ g of plant material was extracted with methanol and ethanol (80/20) for 8 hours in approximately 300ml (80 (solvent)/20 (water)).the extracts obtained (m_2) were filtered and evaporated (MeOH Ext. and EtOH Ext.).The yield of the extracts is calculated by the following relationship:

$$R\% = \frac{m_2}{m_1} \times 100$$

The MeOH Ext. recovered from the previous extraction is mixed with 30 ml of boiling distilled water and left at room temperature overnight. After filtration, successive liquid-liquid type extractions were performed using ethyl acetate and n-butanol., three other extracts are obtained; ethyl acetate extract (AcEth Ext.), butanol fraction (ButOH Ext.) and extract of the remaining residual phase (Ph.Res Ext.) (Fig. 2). The yields of these extracts are calculated by the following relationship:

$$R\% = \frac{m_3}{m_2} \times 100$$

m_3 : mass of the extract obtained

m_2 : mass of the MeOH Ext

2.4. Determination of total phenols

In a 100 ml volumetric flask, a quantity of extract (to obtain a blue color) is mixed with 1.5 ml of Folin-Ciocalteu reagent (10%) and 1.5 ml of sodium carbonate (Na_2CO_3) to 7.5% (m/v). Then the flask is made up of distilled water. The whole is left for two hours at room temperature, and the reading is taken against a blank using a spectrophotometer (UV mini-1240) at 765 nm. The calibration curve is performed in parallel under the same operating conditions using gallic acid as a positive control. The results are expressed in milligrams equivalent to gallic acid per gram of extract (mg GAE/gE) [10].

2.5. Determination of total flavonoids

In a 50ml volumetric flask, 100 μ l of each extract is mixed with 20ml of distilled water, after 5min, 100 μ l of 10% (m/v) aluminum trichloride (AlCl_3) is added. The solutions were adjusted to 50ml with pure methanol, shaken immediately and kept in the dark for 30min at room temperature and the reading is taken against a blank using a spectrophotometer (UV mini-1240) at 333 nm. The calibration curve is carried out in parallel under the same operating conditions using quercetin as a positive control. Results are expressed in milligrams of quercetin equivalent per gram of extract (mg GAE/gE) [11].

2.6. Dosage of condensed tannins

A volume of 3 ml of the vanillin/methanol solution (4% m/v) is added at different concentrations prepared from the catechin solution (2 mg/ml). Everything is mixed manually. Then, a volume of 1.5ml of concentrated hydrochloric acid is added to each concentration. The mixtures obtained are left to react at ambient temperature for 20 min. Absorbance is measured at 499nm against a blank using a spectrophotometer. The number of condensed tannins in our samples (extracts) was carried out following the same procedure used to plot the calibration curve, replacing the catechin with our samples. The concentration of tannins is estimated in milligrams of catechin equivalents per gram of dry matter weight from the calibration curve [11].

2.7. Analysis and identification of the chemical composition of phenolic extracts by HPLC/ UV/MS.

To explore the diversity of phenolic compounds present in the different extracts from MAPs, it is essential to use a method that allows them to be separated, quantified, and identified. High-performance liquid chromatography (HPLC) coupled with UV-Visible detection and a mass spectrometer has been developed. The column used in this method is of the C18 reverse phase type. The mobile phase for the elution of the

molecules is a mixture of solvents formed from acetonitrile and a solution of phosphoric acid with a concentration of 0.05 M. The solvent gradient in this experiment is as follows: 2% acetonitrile (isocratic) 0–3 min, 2–30% acetonitrile in phosphoric acid (linear gradient) 3–19 min, 30–80% acetonitrile in phosphoric acid (linear gradient) 19–23 min, 80% acetonitrile in phosphoric acid (isocratic) 23–28 min, 80 – 2% acetonitrile in phosphoric acid (linear gradient) 28–32 min, 2% acetonitrile (isocratic) 32–40 min. The flow rate is 1.5 mL/ min. The injection volume is 20 µL. Detection in UV is by scanning in the wavelength range of 200–400 nm and then at the acquisition wavelength of 472 nm.

Electrospray is the method used for the ionization of molecules in mass spectrometry. The standards used are: gallic acid, ellagic acid, tannic acid, quercetin and coumarin.

The analysis of the eluted compounds is carried out on the one hand by comparing the retention times of the various peaks of the extracts with the retention times of the peaks corresponding to the standards, then by analysis of the mass spectra of the eluted molecules.

2.8. Antioxidant Activity

2.8.1. Scavenging test of free radical DPPH*

The antiradical activity of the various extracts of the studied plant is established by the method based on DPPH* (1,1-diphenyl-2-picrylhydrazyl) as a relatively stable radical. 200 µL of different concentrations of the samples are placed in the presence of 2.8 ml of the ethanolic solution (2.4 mg of DPPH*/100 ml, the absorbance at 517 nm of this solution is between 0.6 and 0.7). The tubes are then placed in the dark at room temperature for 30 minutes, then the absorbance is measured at 517nm. These same concentrations were prepared with ascorbic acid (vitamin C) and butyl hydroxyanisole (BHA) to serve as positive controls. Also, a blank was made with absolute ethanol alone.

The results were expressed as a percentage reduction in DPPH* (AA%).

$$AA\% = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

AA%: Percentage of antioxidant activity.

A_{control} : absorbance of the solution containing only the solution of the DPPH* radical.

A_{sample} : absorbance of the solution of the samples to be tested in the presence of DPPH*.

The values of IC_{50} were determined graphically by linear regression, The IC_{50} is the concentration of the test sample necessary to reduce 50% of the DPPH radical.

The 50% effective concentration (EC_{50}) was expressed in terms of the concentration of sample extract used for the test (mg/ml) and the amount of extract relative to the initial amount of DPPH (mg/mg

DPPH).

$$CE_{50} \left(\frac{\text{mg}}{\text{ml}} \right) = \left[\frac{CI_{50} \left(\frac{\text{mg}}{\text{ml}} \right)}{\text{Concentration of DPPH} \left(\frac{\text{mg}}{\text{ml}} \right)} \right]$$

For rational reasons of clarity, the antiradical power (ARP) was determined as the reciprocal value of the effective concentration EC_{50} [12].

$$ARP = \left[\frac{100}{CE_{50}(\text{mg / ml})} \right]$$

The correlation between the levels of phenolic compounds and the methods of antiradical activity was determined by Pearson's coefficient using EXCEL (2013).

2.8.2. Iron reduction test: FRAP (Ferric reducing antioxidant power)

The power of phenolic extracts to reduce ferric iron (Fe^{3+}) present in the potassium ferricyanide complex to ferrous iron (Fe^{2+}) is determined according to the method described by [13].

In test tubes, 0.5ml of the extracts at different concentrations (same concentrations used in the DPPH test), is mixed with 2.5ml of a 0.2M phosphate buffer solution (pH = 6.6) and 2.5ml of a solution of potassium ferricyanide $K_3Fe(CN)_6$ to 1%.

The whole is incubated in a water bath at 50°C for 20 minutes. Then 2.5ml of 10% trichloroacetic acid is added to stop the reaction. The whole is centrifuged at 3000 revolutions for 10 min. then 2.5 ml of the supernatant from each concentration is mixed with 2.5 ml of distilled water and 0.5 ml of the 0.1% aqueous $FeCl_3$ solution. The absorbance of the reaction medium is read at 700nm against a similarly prepared blank, replacing the aqueous extract with distilled water which makes it possible to calibrate the device (UV-VIS spectrophotometer). The positive control is represented by solutions of standards (Ascorbic acid, BHA, and BHT) whose absorbance was measured to compare the results. An increase in absorbance corresponds to an increase in the reducing power of the extracts tested. The graph of the change in absorbance as a function of the extract concentration was used to determine the concentration corresponds to an absorbance of 0.5 ($EC_{0.5}$) by linear regression.

2. Results And Discussion

3.1. Phytochemical screening

To detect the different classes of secondary metabolites present in *T. articulata*, we performed phytochemical tests on their organic and aqueous extracts. These tests have the advantage of revealing the chemical groups present in the plant material, which allows us to have a better appreciation of the proven and potential biological activities of the species in our study. The main chemical groups, identified by these tests (Table 1), were the polyphenols (flavonoids and tannins), osteoids, holosides, sterols, triterpenes, and mucilages.

For polyphenols, both gallic and catechic tannins were detected in *T. articulata* extracts. As for flavonoids, they exist in several forms, flavones, dihydroflavonols, flavonols, flavanonols, and anthocyanins. The characterization tests have shown the presence of leucoanthocyanins and free flavonoids and the absence of anthocyanins. The tests using the reagents of Mayer and Dragendorff allowed the demonstration of the richness of *T. articulata* extracts in alkaloids. The presence of this chemical family in *T. articulata* may increase the possibility that it exhibits important biological properties. Indeed, we can cite, for example, the alkaloids shown in *N. latifolia* gave it antimalarial and antimicrobial activity [14]. Also, the alkaloids (vincristine and vinblastine) extracted from the Madagascan periwinkle (*Catharanthus roseus*) are used in cancer treatments. However, some studies show that alkaloids, although therapeutically interesting, can constitute a significant risk of toxicity (Stegelmeier et al., 2016; [16]. Other tests of characterization have shown the presence of sterols and terpenes, oses, holosides, and mucilages while the carotenoids and Coumarins are absent. On the other hand, given that the decoction is the preferred method of preparation of traditional remedies, and based on the results of phytochemical tests, the remedies used by the populations seem to be rich in polyphenolic compounds, oses, holosides, and mucilages. These compounds with various activities are also known for their effects on the digestive system and pain relief [17]. This could help explain the fact that most plants have a use in traditional medicine to treat digestive disorders and abdominal pain. Also, despite the possible abundance of alkaloids in a plant, they could be found in low proportions in traditional remedies due to their insolubility in water. So, the risk of exposure to the toxic effects of alkaloids can be reduced. Polyphenolic compounds being the most represented in *T. articulata* and probably the most important in traditional remedies, we have advanced our research by performing assays of these polyphenolic compounds in the studied species.

Table 1
Phytochemical Screening of *T. articulata*

Phytochemical screening of <i>T. articulata</i>			<i>T.articulata</i>
Chemical family			
Alcaloïds	Mayer		+
	Dragendorff		+
Polyphenols	Tannins	Gallic tannins	-
		Catechic tannins	+++
	Flavonoïds	Anthocyanes	-
		Reaction of Cyanidine	+++
		Leucoanthocyanes	+++
free anthracenic derivates			-
combinedanthracenicderivates	O-heterosides		-
	C-heterosides		-
Sterols et triterpenes			+++
Mucilages			+++
Saponins			-
Cardiotonic Heterosides			-
Carotenoïds			-
Oses and holosides			+
Quinones			-
+++ : Reaction strictly positive, + : reaction positive, -: reaction negative			

3.2. Yields of phenolic compounds

The solid-liquid extraction of the phenolic compounds from *T. articulata*, carried out by soxhlet using hydroalcoholic solutions: methanol/water and ethanol/water at 80% allowed obtaining two crude extracts for each sample, these are hydroethanolic extract (MeOH Ext.) and hydroethanolic extract (EtOH Ext.) respectively. The results are summarized in Table 2. The yield of hydromethanolic extract of *T. articulata* was stronger ($38.36 \pm 0.31\%$) than that observed in hydroethanolic one ($17.23 \pm 0.45\%$). These results are in agreement with those carried out by [18], who found that the effective solvent for extracting polyphenols, with the three used methods (maceration at room temperature, hot infusion and microwave), was methanol followed by ethanol and water. Moreover, the extraction of methanol gives a wide range of phenolic compounds, including anthocyanins, phenolic acids, catechins, flavanons, flavanols and procyanidins [19] [20]; Ross et al., 2009). For fractionation of crude extract (MeOH Ext.) of *T. articulata*,

the results showed that the extract from the residual phase exhibited the highest yield ($40.72 \pm 0.81\%$) followed by BuOH Ext. ($23.81 \pm 0.09\%$), then AcEth Ext. ($18.19 \pm 0.11\%$). Indeed, the highest yield observed for ResPh Ext. allowed us to conclude that there are chemical compounds that are not soluble in either ethyl acetate or butanol. Differences in the extraction efficiency of various solvents have been attributed to their polarities [22]. So, the extraction yield depends on the polarity as well as the variety of intermolecular interactions or the selectivity of the solvents. It is difficult to compare the results with those in the literature, the yield is only relative and depends on the method and conditions under which the extraction was carried out.

Table 2
Yields of extracts by Soxhlet of the *T.articulata*

	EtOH Ext.	MeOH Ext.	AcEth Ext.	BuOH Ext.	Ph.Res Ext.
<i>T. articulata</i>	17.23 ± 0.45	38.36 ± 0.31	18.19 ± 0.11	23.81 ± 0.09	40.72 ± 0.81

3.3. Total Phenols, Flavonoids And Condensed Tannins Content

The contents of total phenols, total flavonoids and condensed tannins in extracts of *T. articulata* are expressed in mg equivalent of gallic acid, quercetin and catechin per gram of extract (mg EGA /g E) respectively (Table 3). AcEth Ext. recorded the highest content of total phenols, i.e. 654.69 ± 0.33 mg EGA /g E, followed by BuOH Ext. (551.21 ± 0.41) mg EGA / g E, then MeOH Ext. (458.95 ± 0.19) mg EGA/ g E, and finally the ResPh Ext. and EtOH Ext. (144.52 ± 0.25) and 96.71 ± 0.08 mg EGA / g E respectively. Compared to other studies, the aqueous extract of *T. articulata* was characterized by the presence of phenolic compounds with a content reaching 175.67 ± 10.21 μ g EGA / mg MS [23]. The *T. articulata* hydroethanolic extract obtained by soxhlet from Tunisia reached a total phenol content (TPC) of about 38.1 ± 0.6 mg EGA/ g DM [24]. Likewise, the acetone extract of Tunisian *T. articulata* recorded a TPC of 32.47 mg EGA / g DM [25]. The EtOH Ext. and MeOH Ext. of *T. articulata* from Algeria get TPCs around 185 and 156 μ g EGA / mg E respectively [26]. According to results of flavonoids dosage, the MeOH Ext. is displayed to be the richest in flavonoids among all tested extracts with a content (FC) of 20.31 ± 0.02 mg eq of quercetin EQ / g Extract, followed by EtOH Ext. (17.98 ± 0.13 mg EQ/ g E) then AcEt Ext. (16.71 ± 0.03 mg EQ / g E). The FC of MeOH Ext. is lower than that observed in MeOH Ext. (37.14 μ g EQ/ mg MV) from *T. articulata* leaves collected in southern Morocco[27]. Also, [23] found that the aqueous extract of *T. articulata*, from the Ait Issi Ihahan region (Morocco), recorded a FC about 11.78 ± 0.30 μ g EQ / mgE which is greater than that obtained in our study (2.37 ± 0.21 mg EQ / g of extract). Compared to our EtOH Ext., that from Algeria is richer in flavonoids (36.70 mg EQ/ g E) [28]. The *T. articulata* AcEt Ext. is completely free from condensed tannins. It can be inferred that ethyl acetate is not a suitable solvent for the extraction of condensed tannins.

Table 3
Polyphenol, flavonoids and condensed tannins of extracts of *T. articulata*

Extracts	Polyphenols (mg EGA/gE)	Flavonoids (mgEQ/gE)	condensed tannins (mg EC/gE)
EtOH Ext.	96.71 ± 0.08	17.98 ± 0.13	12.09 ± 0.82
MeOH Ext.	458.95 ± 0.19	20.31 ± 0.02	15.97 ± 0.74
AcEth Ext.	654.69 ± 0.33	16.71 ± 0.03	
BuOH Ext.	551.21 ± 0.41	8.75 ± 0.92	25.58 ± 0.14
Ph.Res Ext.	144.52 ± 0.25	2.37 ± 0.21	27.72 ± 0.11

3.4 Identification of polyphenols in the MtOH Ext. from *T. articulata* by high-performance liquid chromatography coupled with mass spectrometry (HPLC / UV-ESI-MS)

Chromatographic profiles (Fig. 3) illustrate the peaks of phenolic compounds from *T. articulata* MeOH Ext. and standards and their retention times (RT) and relative abundances. Following the analytical and spectral data (chromatograms and mass spectra), we detected and identified seven compounds whose names and raw formulas are presented in Table 4. The MeOH Ext. chromatogram from *T. articulata* reveals the presence of the majority peaks which extend between 19.30 and 27.78 min. The analysis of the MS and the comparison with the results reported in the literature allowed determining the existence of molecules of the flavonoid type O-glycosides having ions: a pseudo-molecular ion $[M + H]^+$ and fragments $[M-X + H]^+$ corresponding respectively to a loss of a hexose (X: 162) or of rhamnose (X: 146) or both at the same time rutinoside (X: 308). The most important compounds are Catechin, gallocatechin, Myricetin-rhamnose, Quercetin-3-o-Rhamnoside and kampferol-deoxyhexose. These compounds were previously described in *T. articulata* extract, studied by [29] who identified nine compounds: (Epi) catechin dimer type B, (+) - catechin, (-) - Epi catechin, Myricetin-O-pentoxyl-O-hexoside, Myricetin-3-O rutinoside, Myricetin-3-O glucoside, Myricetin-3-O rhamnoside, Quercetin-3-O rhamnoside and Kaempferol-3- O rhamnoside. In addition, [30] have identified seven compounds which are: Catechine, Myricetin-hexose, Myricetin-rhamnoside, Quercetin-3-O-glucoside, Kaempferol-hexose, Quercetin-3-O-rhamnoside Kaempferol-deoxyhexose. Likewise, (+) - catechin and (-) - epicatechin have been found in extracts of *T. articulata* obtained by decoction (aqueous extract) and Soxhlet (ethyl acetate and methanolic extracts) [31]. Indeed, the phytochemical analysis of this extract revealed the presence of a certain number of polyphenolic compounds (flavonols, flavones, flavan). Moreover, Myricitrin is a flavonol known for its numerous pharmacological effects [32], it is used as a supplement in functional foods, cosmetics and drugs due to its strong antioxidant activity [33] [34], antinociceptive, anti-inflammatory [35] and antiallodynic activity [33].

Table 4
Identification by HPLC-MS of polyphenols in MtOH Ext. of *T. articulata*

N°	Name	Masse	Ion M/Z	Fragments	Area %
1	Catechin	291	292	291	4.25
2	B-type (Epi) catechin dimer	587	588	578, 453, 288	9.35
3	Gallocatechin	306	307	306	6.89
4	Myricetin-hexose	480	481	480	4.67
5	Myrcetin-rhamnose	465	466	465,319	14.39
6	Quercetin-3-o-Rhamnoside	449	450	449,303	4.98
7	kampferol-deoxyhexose	433	434	433,287	6.27

3.5. Antioxidant activity

3.5.1. DPPH free radical scavenging test

The antiradical activity of *T. articulata* extracts and the reference standards (ascorbic acid and BHA) was carried out by the DPPH method. The results obtained are shown in Fig. 4. From obtained data, we see that MeOH Ext. and EtOH Ext. has inhibition percentages of about 93.94% and 91.99% respectively for a concentration of 472 µg / mL, these percentages stand out by being greater than the ascorbic acid and BHA. Indeed, AcEth Ext., BuOH Ext. and Res Ph Ext. have significant anti-free radicals on DPPH free radicals with inhibition percentages of 82.71% 82.71%, and 85.87% respectively while, those of ascorbic acid and BHA are of the order of 90.49% and 89.29% respectively for a concentration of 472µl / ml. According to the results about inhibitory concentrations of 50% (IC₅₀) values (Table 5), the EtOH Ext. has moderate antioxidant power (IC₅₀ = 70 ± 0.07 µg / ml) followed by MeOH Ext. (IC₅₀ = 90,68 ± 0.32µg / ml), AcEth Ext. (156.36 ± 0.04µg / ml), BuOH Ext. (231.37 ± 0.14µg / ml) and finally Ph.Res Ext. (104.01 ± 0.05µg / ml). These values are much higher than those of ascorbic acid (31.44 ± 0.24µg / ml) and BHA (46.25 ± 0.31µg / ml).

Table 5
The IC₅₀ (µg/ml) of the reference standards and extracts of *T. articulata*

Extracts	Ascorbic. A	BHA	EtOH Ext.	MeOH Ext.	BuOH Ext.	AcEt Ext.	Ph.Res Ext.
IC ₅₀	31.4 ± 0.24	46.25 ± 0.31	70 ± 0.07	90.68 ± 0.32	231.37 ± 0.14	156.36 ± 0.04	104.01 ± 0.05

3.5.2. Iron reduction test: FRAP (Ferric reducing antioxidant power)

The antioxidant activity of extracts of *T. articulata* was also evaluated using the FRAP method. The results (Fig. 5) show that the reducing power of the extracts is dose-dependent (concentration-dependent). At a concentration of 0.4 mg/ml, the reducing power of BuOH Ext. is much greater (OD = 1.50) compared to MeOH Ext., AcEth Ext. and Ph.Res Ext., but clearly lower than that of ascorbic acid (OD = 1.9), BHA (OD = 1.88) and BHT (OD = 1.81). The concentrations corresponding to the optical density of 0.5 are described in Table 6. the BuOH Ext. has a relatively interesting EC_{0.5} (100 ± 0.65 µg/ml), compared to other extracts MeOH Ext. (225.65 ± 0.14 µg /ml), AcEth Ext. (470 ± 1.52 µg/ml) and Ph.Res Ext. (980 ± 0.57 µg /ml). Furthermore, the positive controls of Ascorbic acid, BHA and BHT recorded EC_{0.5} values around 19 ± 0.35 µg /ml, 46.25 ± 0.78µg /ml and 75 ± 0.92 µg /ml respectively. These results agree with those reported by [23], who found that the aqueous extract from *T. articulata* gets IC₅₀ = 27.38 ± 0.02 µg / ml and 47.12 ± 0.15 µg / ml by the DPPH and FRAP methods respectively. In addition, the study performed by [29] showed that the aqueous extract, ethyl acetate extract, butanolic extract from leaves of Algerian *T. articulata*, exhibit significant antiradical power with IC₅₀ = 12.7; 4.5 and 8.7 µg /ml for aqueous extract, ethyl acetate extract, butanol extract respectively.

Table 6
The EC0.5 of extracts of *T. articulata* and reference standards

Extracts	EC _{0.5} (µg/ml)
Ascorbic acid	19 ± 0.35
BHA	46,25 ± 0.78
BHT	75 ± 0.92
EtOH Ext.	104,12 ± 1.02
MeOH Ext.	225,65 ± 0.14
AcEt Ext.	470 ± 1.52
BuOH Ext.	100 ± 0.65
Ph.Res Ext.	980 ± 0.57

3.5.3. Correlation between the content of phenolic compounds and the antioxidant activities of the extracts

All the obtained results from the assays of phenolic compounds and antioxidant activities (DPPH and FRAP) indicate that the extracts that recorded significant antioxidant potential are very rich in polyphenols and flavonoids. So, there are proportional relationships between antioxidant activities and total polyphenols. To better characterize these possible relationships between the different variables, we calculated the correlation coefficients (Table 7). Depending on the results of the antioxidant activity evaluated by the two methods DPPH and FRAP, it appears that there is a weak positive correlation for the

extracts of *T. articulata*, ie $R^2 = 0.23$. This difference in antioxidant activity between both methods could be attributed to the difference in compounds of the extracts which intervene by different types of action mechanisms. The correlation established between the FC of the *T. articulata* extracts and the antioxidant power determined by the DPPH test is strongly positive ($R^2 = 0.82$). Similarly, a weak positive correlation ($R^2 = 0.25$) is observed between the reducing power and the FC. However, the correlation between the antioxidant power and the TPC is weakly negative, which shows the richness of these extracts by other phenolic compounds which do not have antioxidant potential. The antioxidant activity of MeOH Ext. may be due to its chemical profile rich in Catechin, Gallocatechin, Myrcetin-rhamnose, Quercetin-3-o-Rhamnoside, and kampferol-deoxyhexose. The identified phytochemicals have been reported to have excellent efficiency as antioxidants [36] [37] [38] [39] Myrcetin and quercetin, the main flavonols found in our sample, have already been cited as antioxidants by [40] [41]. It should be noted that the TPC still does not correlate with antioxidant activity. This may mean on the one hand that the antioxidant activity is mainly due to the quality of the phenolic compounds in the extracts and not to their amounts. On the other hand, the anti-free radical and reducing powers of the extracts found in this study may not relate to flavonoids and polyphenols, but other chemical groups with antioxidant activity.

		Table 7			
		Linear correlation coefficients (R2) between the content of phenolic compounds and the antioxidant activity of the extracts of the plants studied			
		TPT	TFT	ARP(DPPH)	1/EC _{0.5} (FRAP)
<i>T. articulata</i>	TPT	1	-0.28	-0.77	-0.023
	TFT		1	0.82	0.25
	ARP(DPPH)			1	0.23
	1/EC _{0.5} (FRAP)				1

3. Conclusion

The present study allowed, on the one hand, highlights the richness of *T. articulata* in polyphenols, flavonoids and condensed tannins and on the other hand to show the richness of the hydromethanolic extract, by the HPLC/ MS analysis, in the flavonoid compounds. Moreover, the antioxidant power depends on the quality and the quantity of the flavonoids present in the extract. Similarly, this study confirmed also that the leafy branches of *T. articulata* have excellent antioxidant power and therefore justify their wide use in traditional herbal medicine to treat several diseases.

Declarations

Ethical Approval

No applicable

Competing interest

No applicable

Authors' contributions

Applicable

Funding

No applicable

Availability of data and materials

No applicable

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Figures



Figure 1

Morphological profile of *T. articulate* Vahl

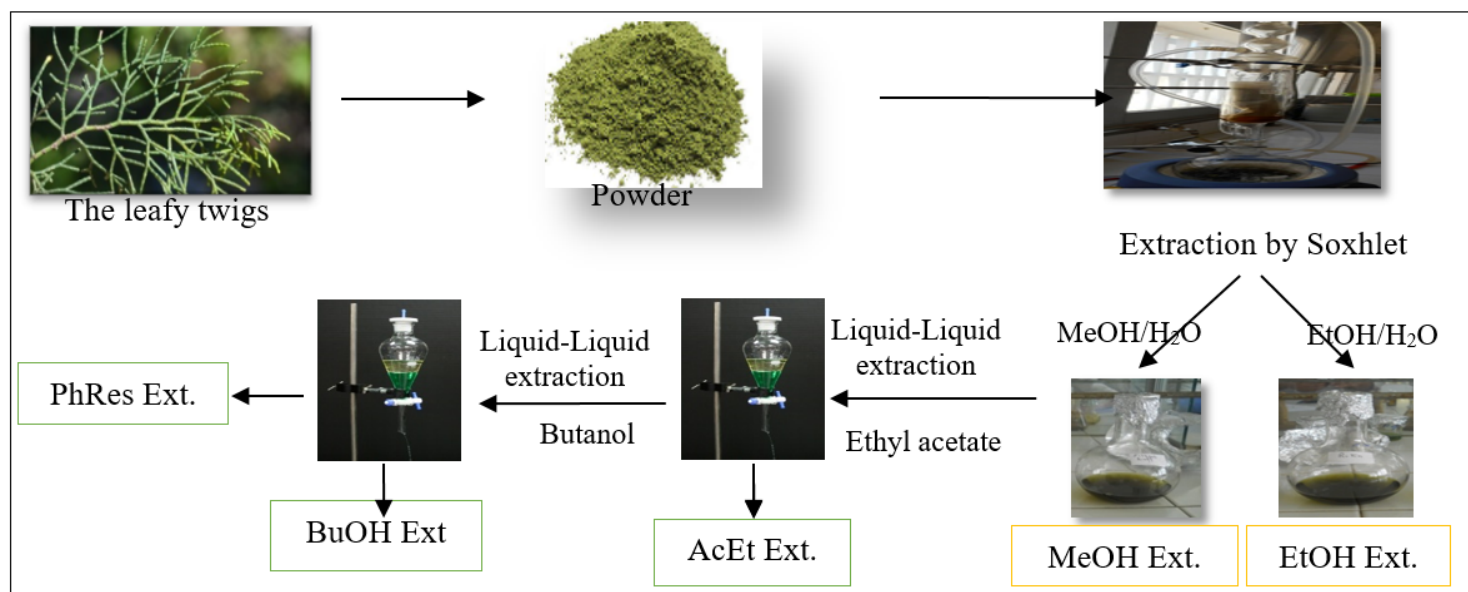


Figure 2

Stages of extraction and fractionation of phenolic compounds

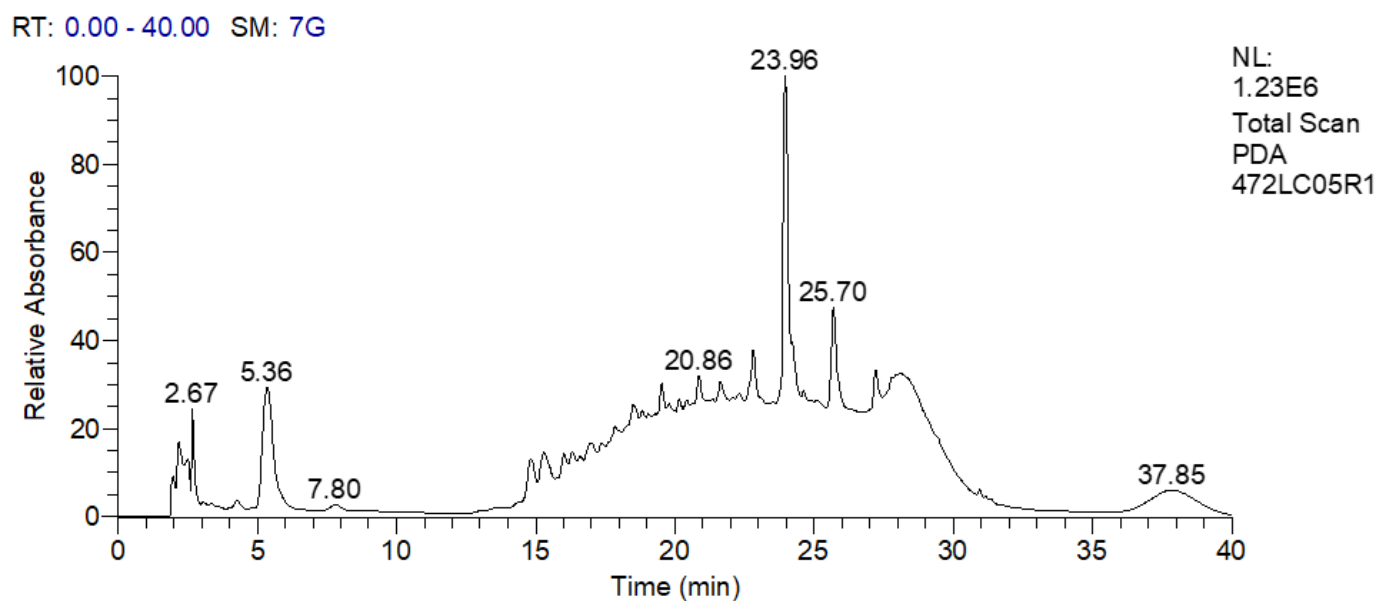


Figure 3

HPLC chromatograms of polyphenols of E.MeOH from *T. articulata*

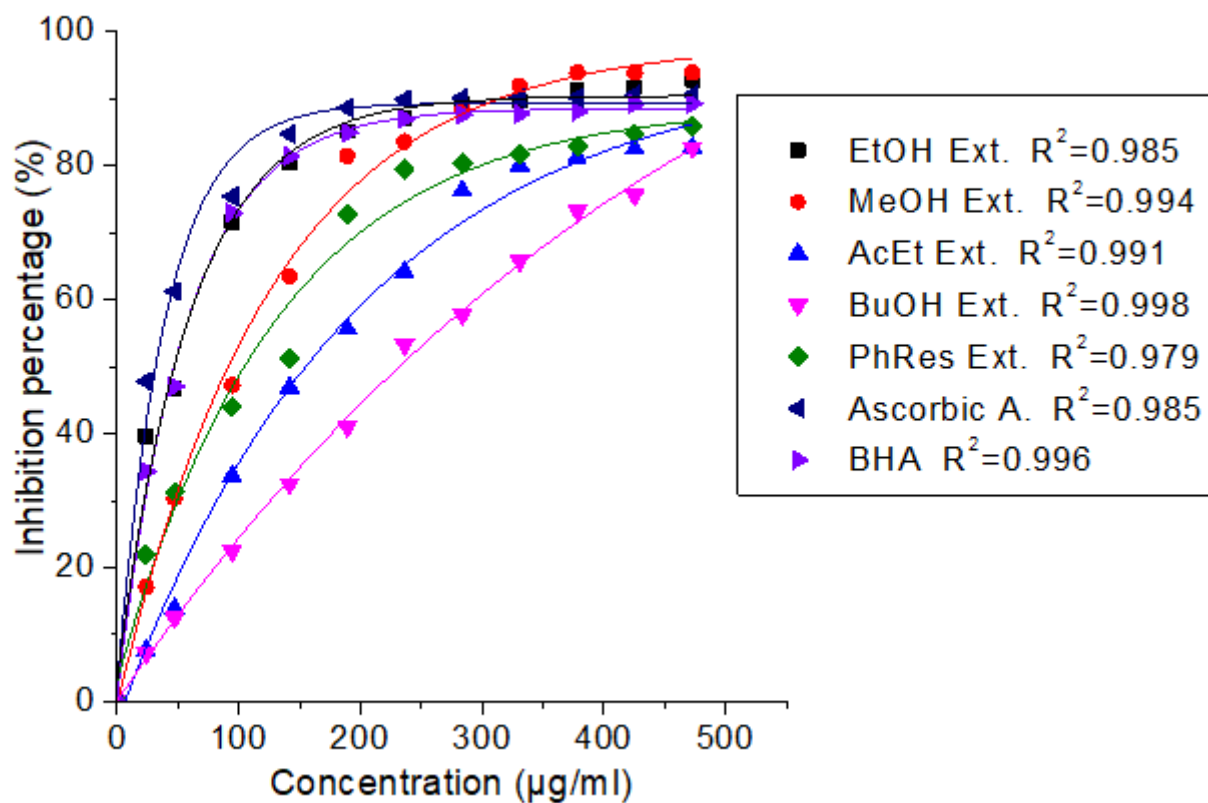


Figure 4

Percentage of inhibition as a function of the concentrations: of the reference standards and the extracts of *T. articulata*.

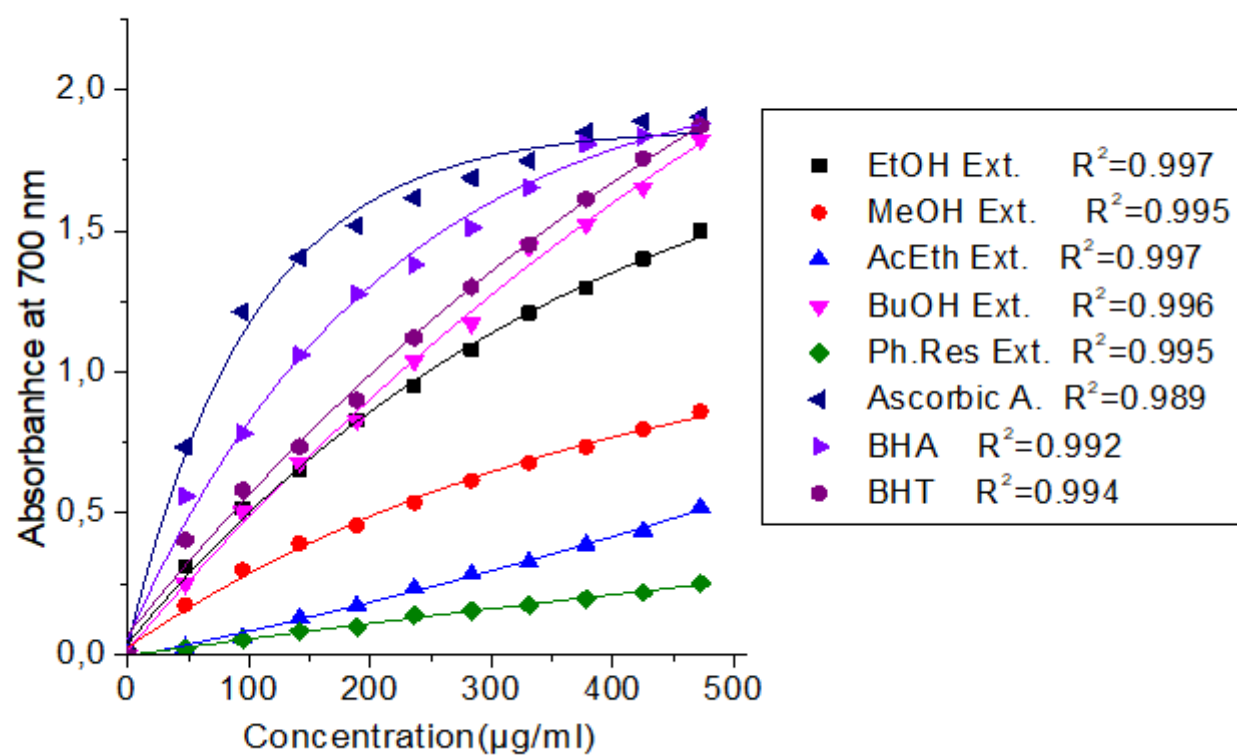


Figure 5

Absorbance as a function of concentrations: reference standards and extracts of *T. articulata*.