



ESTIMATION, CHARACTERIZATION OF PHENOLICS COMPOUNDS AND IN VITRO EVALUATION OF THE ANTIOXIDANT ACTIVITY OF THYMUS FONTANESII BOISS ET REUT EXTRACTS

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This study aimed to investigate the hydro methanolic extract and its fractions of aerial parts from Thymus fontanesii Boiss et Reut for their total phenolic and flavonoid contents, characterization of compounds in hydro methanolic extract and antioxidant activity. The total phenolic and flavonoids were determined by Folin-Ciocalteu and aluminium chloride methods, respectively. The characterization of the crude hydromethanolic extract was done by HPLC UV-DAD, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and β -carotene-linoleic acid tests were used to assess antioxidant activity in vitro. The extracts' total phenolic and flavonoid concentrations ranged from 14.56–237.6 mg gallic acid equivalent (GAE)/g. extract and 1.63–2.27 mg quercetin equivalent (QE)/g extract, respectively where the ethyl acetate fraction has the highest value. The characterization of hydro methanolic extract has revealed the presence of salicylic acid, coumaric acid, rosmarinic acid synergistic acid, naringenin 7-glucoside, and luteolin 7-glucoside. The results of antioxidant tests indicated that for the antiradical test the diethyl ether fraction exhibited stronger antioxidant activity, for the FRAP test methanolic extract was more active than other fractions, at least the chloroform fraction was the stronger protector of β -carotene-linoleic acid system against lipids oxidation. Considering these results T. fontanesii Boiss et Reut aerial part can be used for developing antioxidants, that can act against various oxidative diseases.

Keywords: *Thymus fontanesii*, Antioxidant activity, characterization and total phenolic and flavonoid contents.

INTRODUCTION

Aromatic plants in all forms and their processed products since past times have been widely used as flavourings. However, during the last slight decades, they have been considered for the research of bioactive molecules (antioxidant and antibacterial), and for a good conservation of food from damages caused by the diversity of plant products has been considered as a source of biologically active components, primarily phenolic compounds, due to their various biological qualities^{1&2}.

Thymus genus from the Lamiaceae family, which is one of the high sources of culinary

vegetable, flavouring and medicinal plants, are originating from Europe and Southern Asia, assemble several species with large Mediterranean area distribution, and are a native of the mint family and includes about 100 species^{3&4}. Several *Thymus* species are employed in various parts of the world to treat pulmonary, bronchial, gastric, and urinary infections, and they have several spasmolytic, antitussive, and expectorant properties⁵.

Thymus fontanesii Boiss. et Reut. is an endemic plant that grows predominantly on lawn soil in northern Algeria and Tunisia. It is an annual plant with very strong stems, oblong-lanceolate leaves, and white or pale flowers that is called "zaateur" in Algeria. In traditional

medicine, *T. fontanesii* is used as an antispasmodic, and as a drug for carminative, stomachic, expectorant, antitussive, antiseptic and anthelmintic (gastro-intestinal and cold diseases) ^{6&7}.

Several studies have been reported on the essential oil of *Thymus fontanesii*⁸⁻¹⁰. According to the best of our knowledge, there are a few reports of experimental studies about phenolic content and the antioxidant activity of this species concerning extract or fractions. The goal of this research was to quantify the total phenolic and flavonoid content, as well as the characterization of these compounds, and to examine the antioxidant activity of *Thymus fontanesii* Boiss et Reut hydro methanolic extract and its fractions.

MATERIAL AND METHODS

Chemicals and Equipment

Chemicals

All organic solvents (ethanol, n-hexane, chloroform, ethyl acetate, ether diethyl and *n*-butanol) used in this work are from Sigma Aldrich, Germany, Folin-ciocalteu phenol reagent (Sigma-Aldrich, Switzerland), Sodium Carbonate anhydrous (Honeywell, Fluka, Germany), Gallic acid (Titan biotech LTD., India), Aluminum chloride Hexahydrate (Biochem, Chemopharma, USA), Quercetin (Extrasynthese Genay, France), Vitamin C and butylated hydroxytoluene (BHT) (Honeywell, Fluka, Germany), DPPH (TCI Company, Japan), phosphate buffer (Fluka, chemika Switzerland), potassium ferricyanide (Panreac Quimica Sa), Trichloroacetic acid (Sigma Aldrich, Germany), Iron chloride (Panreac Quimica Sa), linoleic acid, β -Carotene and Tween 40 (Sigma Aldrich, Germany), standards used for HPLC analyze: gallic acid, caffeic acid, syringic acid, rosmarinic acid, coumaric acid, chlorogenic acid, naringenin-7-glucoside, kaempferol, catechin, rutin, luteolin-7-glycoside, quercetin, luteolin, naringenin and apigenin-7-glucoside. (Honeywell, Fluka, Germany).

Equipment

Sensitive balance (Kern and Sohn GmbH, Germany), Rotary evaporator (Büchi, R-200, France), UV-1800 spectrophotometer (Shimadzu, Japan), Centrifuge Germany, (EBA 8, Hettich, Germany), HPLC UV-DAD, Agilent 1260 (Canada)

Plant Sample

Thymus fontanesii aerial parts were obtained in June 2017 during the flowering stage in the Guertoufa area of Tiaret province, west of Algeria. Pr. H. Abdelkrim of the National Institute of Agronomy identified the specimens (INA, El Harrache Algeria). The aerial parts of the plant were cleaned and dried in the open area in the shade at room temperature, before being milled into powder.

Extraction and Fractionate Procedure

The extraction and fractionate was carried out following the method reported by Krimat *et al.*, (2015)². In summary, 10g of the powdered plant material was extracted during 48 hrs using 100 mL of methanol-water (70-30%). Filtration was used to recover the solvent. The extraction procedure was carried out three times. To obtain the dry extract, the mixed filtrates were concentrated under reduced pressure at 40°C using a vacuum rotary evaporator. In the second extraction (fractionating), the crude extract was weighed to calculate the yield of extraction after which it was dissolved in 300 mL of hot distilled water at room temperature for 12 hours, hexane was used to defat the aqueous extract (50 mL, three times), after that it was fractionated with equal volumes using different solvents fractions (diethyl ether, chloroform, ethyl acetate and *n*-butanol, taking 50 mL as the volume of fractionating, three times). The fractions have been dried on anhydrous sodium sulphate, then filtered and concentrated under vacuum in a rotary evaporator to dryness.

Total Phenolic Contents

The spectrophotometric method using the Folin-Ciocalteu assay¹¹ was adapted to quantifying total phenolic contents (TPC) of extracts. In a test tube, an aliquot (0.25 mL) of the extract was mixed with 3.75 mL of distilled water, then 0.25 mL of Folin-reagent Ciocalteu's was added. After allowing 3 minutes for the mixture to react, 0.75 mL of 20% sodium carbonate has been introduced. The tubes' contents were stirred with a tube agitator and heated at 40°C for 40 minutes. At 760 nm, the blue coloration was detected. The following equation, derived from a conventional gallic acid graph, was used to compute the quantities of phenolic compounds: Absorbance= 0.1035 gallic acid ($\mu\text{g/ml}$) + 0.1046 (R²:0.98).

Total Flavonoid Contents

The colourimetric method described by Lamairson and Carnet¹², was used to determine the total flavonoid contents in the plant extracts as follow 1.5 mL of methanol-dissolved 2 percent $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ was added to equal volumes of the diluted extract. After agitation the mixture was leaved 10 minutes at room temperature, the absorbance was measured at 440 nm. The amounts of flavonoid components were determined using the equation below, which was derived from a conventional quercetin graph: Absorbance = $0.2829 \text{ quercetin } (\mu\text{g/ml}) - 0.1155 \text{ (R}^2\text{:0.99)}$.

Characterization of Phenolic Compounds

The hydro methanolic extract was put through to the characterization of phenolic compounds by HPLC-UV / DAD analyses, which were carried out with an Agilent 1260 apparatus equipped with a diode array (DAD) UV detector. The analysis was executed in reverse phase with a column C18 (5 μm , 250 \times 4.6 mm). The temperature was maintained at 22 ± 0.8 ° C. and the injection volume chosen was 5 μL . The solvents used were HPLC grade and the flow rate was fixed at 1 mL/min. The chromatographic conditions consist of solvent system A (0.2% acetic acid dissolved in bi-distilled water) and solvent system B (0.2% acetic acid dissolved in acetonitrile) with the following gradient: 0 min: 95 % A + 5 % B; 30 min: 5 % A + 95 % B. Detection was effected at 270 nm, 320 nm and 370 nm. The phenolic acids and flavonoids contained in hydro methanolic extract analyzed were recognized by comparing the retention times and the UV spectra obtained by those of the standards used (gallic acid, caffeic acid, syringic acid, rosmarinic acid, coumaric acid, chlorogenic acid, naringenin-7-glucoside, kaempferol, catechin, rutin, luteolin-7-glycoside, quercetin, luteolin, naringenin and apigenin-7-glucoside).

Antioxidant Activity

DPPH Radical Scavenging Activity Assay

The scavenging activity of the DPPH free radical was determined using the Braca et al., method¹³. Equal amounts of freshly generated DPPH in methanol solution (0.004 percent w/v) were combined with different methanol dilutions of extract and fractions (5 $\mu\text{g/mL}$ to 1000 $\mu\text{g/mL}$). The reaction mixture was properly mixed before being placed at room

temperature in the dark for 30 minutes. The absorbance was taken at 517 nm and a blank that contained the same amount of extracts but no DPPH was used. As antioxidant standards, ascorbic acid and BHT were utilized. The inhibition of the DPPH free radical in percent (I percent) was calculated using the following equation based on a control reading containing equal quantities of DPPH solution and methanol but no test material: $[(\text{AC}-\text{AS})/\text{AC}] \%$ inhibition = $[(\text{AC}-\text{AS})/\text{AC}] \%$. The control reaction absorbance is AC, while the sample absorbance is AS. Using the graph of scavenging effect percentage versus extract concentration, the extract concentration providing 50 % inhibition (IC_{50}) was calculated.

Reducing Power Assay

The Oyaizu method¹⁴ was used to determine the plant's reduction power (extract and fractions). In 1 mL of distilled water, different amounts of plant extracts were mixed with potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$] (2.5 mL, 1 %) and phosphate buffer (2.5 mL, 0.2 M, pH 6.6). 2.5 mL of 10 % trichloroacetic acid was added to the mixture after 20 minutes of incubation at 50°C, it was then centrifuged at 3000 rpm for 10 minutes. The solution's supernatant (2.5 mL) was combined with both distilled water (2.5 mL) and FeCl_3 (0.5 mL, 0.1 %). The absorbance at 700 nm was measured using a UV-Vis spectrophotometer against a blank. As standards, ascorbic acid and BHT were utilized. The $\text{EC}_{0.5}$ value ($\mu\text{g mL}^{-1}$) represents the effective concentration at which the absorbance was 0.5 for power reduction. The reaction mixture's increased absorbance indicates the increasing of reducing power.

β -Carotene/Linoleic Acid Bleaching Assay

The ability of various extracts to minimize the oxidative discoloration of β -carotene in an emulsion phase was assessed using the β -carotene–linoleate model system, as described in ¹⁵. In 10 mL of chloroform, 2 mg of β -carotene was dissolved (HPLC grade). In a round-bottomed flask containing 20 mg linoleic acid and 200 mg Tween 40, 1 mL of this solution was placed. A vacuum evaporator was used to entirely evaporate chloroform. The remaining was then diluted gradually with 50 mL of distilled water and vigorously agitated to make a stable emulsion. A total of 4.8 mL of the produced emulsion was transferred to test

tubes containing 0.2 mL of the extract (2 mg/mL). The tubes were stirred before being incubated for 120 minutes at 50°C in a water bath. A spectrophotometer was used every 30 minutes for 120 minutes to measure the zero-time absorbance (A0) at 470 nm. In a similar manner, a blank reagent was prepared, but without the inclusion of β -carotene. As standards, ascorbic acid and BHT were utilized. The bleaching rate (R) of β -carotene was calculated according to first-order kinetics, as described in Al-Saikhan *et al.*,¹⁶

Statistical Analysis

All of the experiments were done in triplicate. The data were presented as means with standard deviations. A one-way analysis of variance (ANOVA) test followed by a Student's test was used to assess differences. At $p < 0.05$, differences were considered significant. Analysis of variance (ANOVA) was used to determine the correlations between approaches, which were then quantified in terms of the correlation factor.

RESULT AND DISCUSSION

Results

Total Phenolics and flavonoid Contents

Regarding the importance of phenolics contents as bioactive products having a wide

spectrum of biological activities, the quantification of phenolics, and flavonoid contents were assessed. According to the quantification results, it seems that the ethyl acetate and diethyl ether fractions give higher contents than other fractions (*n*-butanol, Hydromethanolique, and chloroform), also the flavonoids contents are lower than polyphenolics.

Table 1 shows the total phenolic content of *T. fontanesii* crude extract and generated fractions; The results revealed that phenolic content varied significantly depending on the composition of the solvent ($p < 0.05$), and 237.6 ± 1.12 mg of GAE/g, as a height content obtained by the ethyl acetate fraction followed by diethyl ether fraction (162.21 ± 7.38 mg GAE/g) and *n*-butanol (78.46 ± 3.391 mg GAE/g. extract). However, the hydro-methanol crude and chloroform fractions showed weaker polyphenol contents (46.88 ± 3.271 and 14.56 ± 0.894 GAE/g extract) respectively.

The same case for the flavonoid contents, where the ethyl acetate and diethyl ether fractions have as higher content, followed by hydromethanolic crude extract with 2.27 ± 0.029 , 2.11 ± 0.041 and 1.99 ± 0.01 mg QE/g, respectively; while *n*-butanol and chloroform fractions showed the lowest amounts with 1.67 ± 0.074 , 1.63 ± 0.017 mg QE/g. extract (Table1).

Table 1: Total phenolic, flavonoid contents (mean \pm SD) of extracts from *T. fontanesii*.

Extracts	Total phenolic contents ^{a,b}	Total flavonoid contents ^{a,c}
Hydro methanolic crude	46.88 ± 3.27	1.99 ± 0.01
<i>n</i> -butanol fraction	78.46 ± 3.39	1.67 ± 0.07
Ethyl acetate fraction	237.6 ± 1.12	2.27 ± 0.03
Diethyl ether fraction	162.21 ± 7.38	2.11 ± 0.04
Chloroform fraction	14.56 ± 0.89	1.63 ± 0.02

^aEach result is shown as a mean standard deviation ($n = 3$). ^bTotal phenolic content was reported as mg gallic acid equivalents/g dried extract, while total flavonoid^c content was expressed as mg quercetin equivalents/g dried extract. The total phenolic and total flavonoid contents do not differ significantly ($p > 0.05$).

Tentative Phenolics Compounds Profile

The phenolic profile of *T. fontanesii*, obtained through hydro methanolic extraction and recorded at 320, enabled us to detect seven compounds, two flavonoids, and five phenolic acid derivatives. As for the phenolic acids: tannic acid, synergistic acid, rosmarinic acid, and coumaric acid. For flavonoid, we have detected kaempferol, luteolin 7-glucoside and Vanillin.

Antioxidant Activity

DPPH Assay (Radical Scavenging Activity)

As shown in table 2, the values of per cent DPPH radical scavenging activities of extract and its fractions of *T. fontanesii* were comparable ($P < 0.05$), with those of the well-known standards antioxidant such as ascorbic acid and BHT (98.54 %, and 82.36 % respectively), where, ethyl acetate, *n*-butanol, Hydro methanolic extract and chloroform, diethyl ether, The fractions have a high percentage of DPPH scavenging activity. (97.59 %, 93.39 %, 93.33 %, 93.16 % and 93.16 %, respectively) at 0.1 mg/mL.

A lower value of IC_{50} indicates a higher antioxidant activity. As shown in table 2, values were given in the sequence: ascorbic acid < diethyl ether < ethyl acetate < hydro methanolic crude < chloroform < BHT < *n*-butanol (Table 2).

The diethyl ether fraction appeared to be superior to all other extracts examined. ($p < 0.05$), When comparing IC_{50} values obtained for standards (BHT: 72.16 μ g/ml) but very inferior to that of ascorbic acid and α -tocopherol (4 and 9.55 μ g/ml respectively). Even ethyl acetate fraction, Hydromethanolic crude extract and chloroform showed a strong antiradical activity than BHT, although the *n*-butanol fraction had lower activity than other fractions.

Table 2: Antioxidant activities of extracts from *T. fontanesii* and standards measured by different assays.

Plant Extracts	DPPH ^{a,b}	% of inhibition ^{a,c}	Reducing power ^{a,d}	β -Carotene / linoleic acid (%) ^a
Hydro methanolic extract	29.84 \pm 0.81	93.333 \pm 0.47	87.81 \pm 3.23	91.86 \pm 1.18
<i>n</i> -butanol fraction	116.43 \pm 2.43	93.391 \pm 1.55	794.45 \pm 23.65	44.61 \pm 1.30
Ethyl acetate fraction	24.99 \pm 1.41	97.586 \pm 0.36	108.08 \pm 6.44	89.35 \pm 0.70
Diethyl ether fraction	22.55 \pm 0.67	92.414 \pm 0.87	332.62 \pm 2.51	94.86 \pm 1.32
Chloroform fraction	42.64 \pm 1.49	93.158 \pm 0.27	739.05 \pm 17.75	96.39 \pm 1.45
Ascorbic acid	4 \pm 0.1	98.54 \pm 0.25	47 \pm 0.28	11.05 \pm 1.43
BHT	72.16 \pm 0.1	82.36 \pm 0.94	633 \pm 11.5	96.92 \pm 0.51

Controlled factor variance analysis at the 0.05% threshold is highly significant for the set of treatments where the smallest significant difference is still below the mean difference.

^aAll values are represented as average SD (n=3), ^b IC_{50} in μ g/ml =concentration for which the inhibition of DPPH free radical approximated equal to 50%. ^c The inhibition of the DPPH free radical in percent (%). ^d $IC_{0.5}$ in μ g/ml =concentration for which absorbance approximated equal to 0.5. ^e Ant-bleaching activity of β -Carotene / linoleic acid in percent(%).

Reducing Power

Table 2, shows the result of reducing power test, of the various fractions, hydro methanolic crude extracts isolated from *T. fontanesii*, and standard antioxidant presented as $IC_{0.5}$. All of the extracts and standards possessed the ability to reduce iron III. The values $IC_{0.5}$, of ascorbic acid and hydromethanolic extract were the lowers, in comparison to other extracts and the synthetic antioxidants BHT thus, they demonstrated the greatest capacity to decrease Fe III to Fe II and revealed no significant difference ($P > 0.05$). In this assay, even ethyl acetate, ether diethyl and *n*-butanol have shown respectively a better activity of reducing Fe III than BHT. $IC_{0.5}$ values were in the following order: ascorbic acid < hydromethanolic crude < ethyl acetate < diethyl ether < *n*-butanol < BHT < chloroform.

β -Carotene-linoleic Acid Bleaching Assay

According to table 2, all of the *T. fontanesii* extracts and conventional antioxidants were capable of lowering the degradation rate of β -carotene by scavenging linoleate-derived free radicals.

The results of percentages of anti-bleaching activities showed that chloroform fraction had the strongest effects on the inhibition of linoleic acid oxidation (96.39 \pm 1.45 %), and their activity was similar to the activity of BHT ($p > 0.05$). followed by ether diethyl fraction (94.86 \pm 1.32 %) and hydro methanolic extract (91.86 \pm 1.18 %) The descending classification of the extracts and standard antioxidant was: BHT > chloroform > diethyl ether > α -tocopherol > hydromethanolic extract > ethyl acetate > *n*-butanol > ascorbic acid.

Discussion

Most antioxidant activities from plant sources are derived from phenolic compounds¹⁷. Phenolic phytochemicals are regarded to promote good health in part because of their antioxidant and free radical scavenging properties, which protect cellular components from free radical damage. However, their antioxidant capabilities are anticipated to differ due to their distinct chemical structures¹⁸. A number of studies using various methodologies have reported antioxidant activity of thyme volatile extracts, with antioxidant effects attributable to the presence of phenolics¹⁹⁻²¹. Therefore, we will compare with the other species of *Thymus*.

A review of the literature revealed that there are only a few papers on the phenolic flavonoid levels, characterization of components, and antioxidant activity of *T. fontanesii* Boiss et Reut hydro methanolic extract and its fractions, by which to compare our results.

TPC and TFC contents

Previous studies carried out on various extracts of the genus *Thymus*. The total phenol content of *T. fontanesii* hydromethanolic extract (of this study), is higher than ethanolic extracts of *T. daenensis*, *T. kotschyanus*, and *T. pubescens* (hydroethanolic extracts) whose have had ranged between 295.57 to 337.00 $\mu\text{g}/\text{mg}$ extract and TFC ranged between 35.21 ± 2.51 to 50.39 ± 0.75 $\mu\text{g}/\text{mg}$ extract²². Roby *et al.*, 2013, studied *T. vulgaris* TPC of methanol and diethyl ether extracts. They found 8.10 ± 2.00 and 6.15 ± 1.86 (mg GAE/g DM respectively) those results are very low than those of this study (46.88 ± 3.27 to 162.21 ± 7.38 mg EAG/gde respectively)²³.

Kholkhal *et al.*, 2013, studying TPC and TFC of *T. ciliatus ssp coloratus* and of *ssp euciliatus* have found as TPC 64.23 mg EAG/g to 16.36 mg EAG/mg (Aerial and root parts respectively) behind 46.88 ± 3.27 of *T. fontanesii* of this study and 298.2 mg EC/g to 90.75 mg EC/g (Aerial part and root parts respectively) whose higher than of this study²⁴.

Nouasri *et al.*, 2018 have studied *T. hirtus* and *T. lanceolatus* with five extracts (as this

study) theirs finding on TPC and TFC are higher than those of *T. fontanesii* of this study²⁵. A study conducted by Niculae *et al.*, (2019), on *T. marschallianus* Willd, have founded a height amount of TPC and TFC (59.89 ± 0.42 to 61.99 ± 0.31 mg EGA/gdw and 16.69 ± 0.51 to 28.98 ± 0.32 25.48 ± 0.23 mg RE/gdw)²⁶, compared to our finding 46.88 ± 3.27 mg EAG/gde (TPC) and 1.99 ± 0.01 mg EQ/gde. Nabet *et al.*, 2019, studied ethanolic extracts by microwave-assisted extraction from *Thymus fontanesii* from the same province (Tiaret), they found as TPC 227.63 mg EAG/gde, and these results are higher than our finding²⁷.

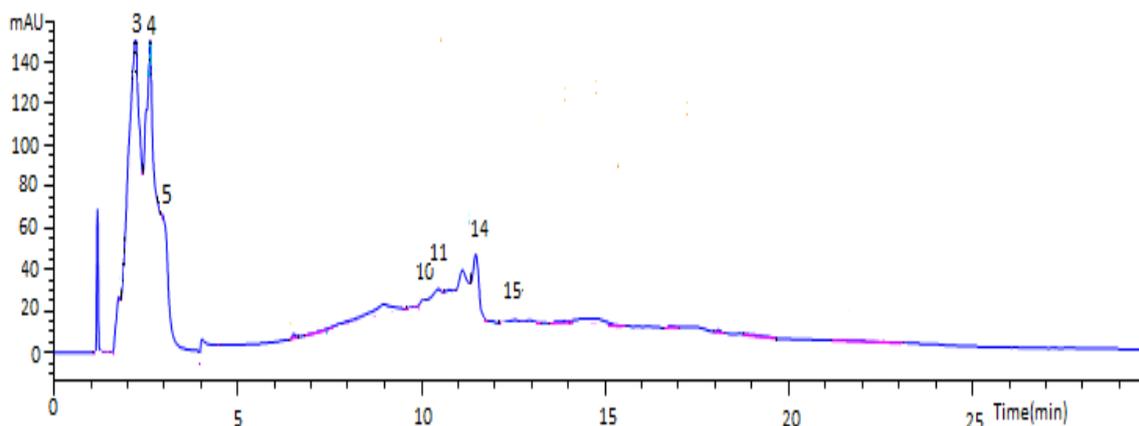
In general, for good extraction of the antioxidant compounds, diethyl ether and/or ethyl acetate are much better than polar solvents²⁸. *T. fontanesii* extracts had a modest quantity of phenolic content, including total phenols and flavonoids, according to the phytochemical investigation. Flavonoids and phenolic compounds are the most important classes of secondary metabolites in plants as plant compounds²⁸, and they are responsible for biological activity.

HPLC analysis

Secondary metabolites such as terpenoids and flavonoids are known to be present in Lamiaceae species²³. As a result, employing high-performance liquid chromatography (HPLC), for qualitative examination of *T. fontanesii* hydromethanolic plant extracts was performed.

Representative chromatograms are shown in Fig. 1. The phenolic profile of *T. fontanesii*, obtained after hydro methanolic extraction, and recorded at 320, let us to identifying seven compounds, four of which were phenolic acid derivatives and three flavonoids. As for the phenolic acids: tannic acid, synergistic acid, rosmarinic acid, coumaric acid and vanillic acid. For flavonoid, we have detected kaempferol, and luteolin 7-glucoside.

We have to compare no methods used in the literature consulted, but the presence of phenolic compounds. Proestos *et al.*, 2005²⁹, in their study of *T. vulgaris* have found fewer and different compounds compared to *T. fontanesii*.



15=coumaric acid, 14=rosmarinic acid, 11=vanillin, 10= luteolin 7-glucoside, 5= synergistic acid, 4= kaempferol, 3=tannic acid.

Fig1: *Thymus fontanesii* hydro-alcoholic extract phenolic profile measured at 320 nm.

The numbers which precede the phenolic compounds and flavonoids are those of the peaks in the chromatograms.

Costa *et al.*, 2012 studied *Thymus lotocephalus* wild., and from in vitro culture, they have found nearly the same profile between the two different sources (caffeic and rosmarinic) acids and flavones (luteolin and apigenin), compared to *T. fontanesii* phenolic identified it's considered low³⁰. for Roby *et al.*, 2013²³, in their study of different extracts of *T. Vulgaris* have found in methanolic extract the presence of different compounds of that found in *T. fontanesii* of this study. Pereira *et al.*, 2013, during their characterization and quantification of phenolic components in *Thymus x citriodorus*, they have found rosmarinic acid also in luteolin-7-O- α -glucuronide. eriodictyol dihexoside with O-glycosides linkages, one quercetagenin dimethyl ether-O-hexoside, two eriodictyol-O-mono-hexosides, one naringenin-O-hexoside, and chrysoeriol-7- β -O-glucoside³¹, thus it's different of what we find in *T. fontanesii*.

Sevindik *et al.*, 2015, have studied the *Thymus praecox sub sp. grossheimii var. grossheimii* and mention the presence of ursolic acid, oleanolic acid, methyl rosmarinate, ethyl rosmarinate, rosmarinic acid, luteolin 5-O- β -D-glucopyranoside, and thymoquinol 2,5-O- β -diglucopyranoside³². Those results are very different qualitatively from those of this study. Öztürk *et al.*, 2015 have studied the phenolic composition and antioxidant activity of the different extracts from two spices of *Thymus longicaulis* growing in Turkey, the results obtained from their study were close to our finding in the number of phenolic compounds³³.

Boutaoui *et al.*, 2018 with *Thymus algeriensis*, have analyzed different extracts of this thyme, maceration, supercritical fluid extraction, and microwave-assisted extraction. And different subfractions of the hydromethanolic extract as chloroform, ethyl acetate, and *n*-butanol. The results demonstrate that a phenolic-rich extract of *Thymus algeriensis* aerial parts was obtained using water extraction facilitated by microwave at 100°C for 15 minutes, and the *n*-butanol extracts were more rich phenolics, they reported the presence of 11 phenolic acids and 5 flavonoids³⁴. Some of what they find, is present in *T. fontanesii* phenolic profile. Nouasri *et al.*, 2018²⁵, with *T. hirtus* and *T. lanceolatus*, have had approximately the same profile with *T. fontanesii* of this study.

Taşkın *et al.*, 2018 have characterized phenolic of *Thymus praecox subsp. skorpilii var. skorpilii*, they found chlorogenic acid, luteolin-7-O-glucoside, 3-O-feruloylquinic acid, quercetin-3-O-hexoside, and apigenin-7-O-glucuronide³⁵, this result is different quantitatively and qualitatively of *T. fontanesii*.

Taghouti *et al.*, 2020, studied *Thymus mastichina* and reported that, the hydromethanolic extract from *Thymus mastichina* contains lower amounts of extractable phenolic compounds compared to other *Thymus* species. However the phenolic profiles of the hydromethanolic extract from *Thymus mastichina* are comparable to the phenolic profiles described in the literature for various *Thymus* species³⁶. This means is close to our finding.

Nabet *et al.*, 2019, in their study of phenolics profile of *T. fontanesii* methanol extract assisted by microwave technical, using HPLC-DAD-ESI-MS/MS, found twenty-six compounds²⁷, all of our findings are present in their results, which is in concordance with our finding. Sobeh *et al.*, 2020, carrying out the analysis of phenolics profile of *T. fontanesii* (ethanol extract) from Ain El-Defla province (west Algeria), by HPLC-PDA-ESI-MS/MS, identified all that we found in this study³⁷.

The solubility of phenolic compounds depends on the chemical nature of the plant tissue and the polarity of the solvent system³⁸. Growing circumstances, harvesting, and processing, among other factors, could explain the differences in phenolic profiles identified in samples from diverse sources, which directly interfere with the quantity of chemical elements and, as a result, their therapeutic effects²⁵.

In general, for good extraction of the antioxidant compounds, diethyl ether and/or ethyl acetate are much better than polar solvents^{39&40}. The attempt of identification and the result obtained was compared with the literature of other species where several studies report the presence of phenolic acid and flavonoid found in *Thymus* genus^{23&41}.

Antioxidant activities

Although different solvents were utilized for extraction, the results obtained by the DPPH radical scavenging activity are roughly in agreement with previous studies on *Thymus* species. Kholkhal and colleagues (2013)²⁴, showed that ethyl acetate fraction of *Thymus ciliatus ssp. coloratus* was more active than *n*-butanol fraction (both aerial parts and roots), 850 to 4500 µg/ml, and 1500 to 6250 µg/ml respectively. But compared to our finding their IC 50 values were higher than those of this study, that fact they are no more active than our fractions.

Bahman N. and Naser E. (2012), have found that ethanolic extract of *T. daenensis*, *T. kotschyanus*, and *T. pubescens* has a good scavenging activity with IC₅₀ value 31.47, 47.22, and 48.58 µg/ml (respectively)²², these results are comparable to our finding. Guesmi *et al.*, (2014) for *T. hirtus ssp. algeriensis*, found as a percentage of ant-DPPH activity between 81 ± 0.26 and 93 ± 0.06%⁴², these results are lower compared to those of this study.

Jabri-Karoui *et al.*, (2012) for methanolic extract of *T. capitatus*, (flowers), found an IC₅₀ of 12 ± 0.06 µg/mL¹, thus the methanolic extract of flowers from *T. capitatus* is very active than of this study. Turumtay *et al.*, (2013) study the stem, leaf, and flowers of *Thymus praecox Opiz subsp. Caucasicus var. caucasicus*, found an IC₅₀ between 55 ± 0.00 to 115 ± 0.02 µg/ml¹, these results are almost lower than those of *T. fontanesii*.

The previous study reported the RP test of thyme spices: Rita *et al.*, 2018, assessed the infusion extract (polar solvent), of *Thymus × citriodorus* (and other spices), they found a concentration of 228 ± 0.003 µg/ml^{4r}. This value is higher than that of hydromethanolic and ethyl acetate (87.81 ± 3.23 and 108.08 ± 6.44 respectively), thus is lower activity than of this study. For *Thymus schimperii Ronniger* (studied by Dessalegn *et al.*, 2015), the EC₅₀ of methanolic extract was 655.5 ± 13.6 µg/ml, which is very higher compared to our finding, so the very low capacity of reducing power^{4s}. But for *Thymus capitatus* the results are porches of ours (380 ± 0.06 µg/ml)¹.

Compared to other thyme, Kholkhal *et al.*, (2014) in their study of two species of thymes *T. ciliatus ssp. coloratus* and *ssp. eucilatus* had obtained a percentage of activity for hydro methanolic extract and fractions: ethyl acetate and/or *n*-butanol values not exceeding 73%²⁴. Guesmi *et al.*, (2014), with the methanol extract of *T. hirtus ssp. algeriensis*, had given a percentage inhibition of bleaching of β-carotene in average 52 %⁴². These values are low when compared to the antioxidant activity of *T. fontanesii* extracts reported in this investigation. The hydromethanolics and their fractions of *T. hirtus* and *T. lanceolatus*²⁵, on the other hand, have a higher potential to preserve β-carotene from discolouration than those of *T. fontanesii* found in this investigation. By the results of the tree test conducted in this study, the moderately polar and nonpolar fractions are particularly active, which is owing to their greater quantities of phenolic compounds, some of which were discovered by HPLC UV-DAD and are known for their activities, such as rosmarinic acid^{4s}. (Kim and Lee 2004), which implies that the 3-OH group of the chroman ring has a structurally crucial function in boosting antioxidant activity in compounds including quercetin, kaempferol, luteolin, caffeic, and p-coumaric. These low polar solvents have also

been discovered to facilitate the extraction of terpenes, sterols, and coumarins, all of which are known to have biological functions, particularly an antioxidant effect. This may explain in part the dominance of low polar solvents in the majority of antioxidant tests used in this study ⁴⁷.

Conclusion

In this work, hydro methanolic extract and its fractions of *T. fontanesii* demonstrated potent antioxidants. An average rate of total phenolics which is probably responsible for the antioxidant activity observed. These results will facilitate future studies in identifying, isolating, and characterizing the particular chemical that causes these activities, and assess other biological tests such as anti-cancerous, anti-inflammatory, cytotoxicity... These results indicate that the *T. fontanesii* Boiss et Reut plant can be used for developing antioxidants, that can act against various oxidative diseases.

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نشرة العلوم الصيدلانية جامعة أسيوط



تقدير و تشخيص المركبات الفينولية، و اظهار مختبريا النشاط المضاد للأكسدة
للمستخلص الهيدروكحولي و أجزاءه العضوية لنبات

REUT THYMUS FONTANESII BOISS ET

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ص ب ٩٢ الجزائر

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هدفت هذه الدراسة إلى التحقق من مستخلص الميثانول المائي وأجزاءه العضوية من القسم الهوائية من *Thymus fontanesii Boiss et Reut* لمحتواها الكلي من الفينول والفلافونويد ، وتشخيص المركبات فيها ، ومعاينة النشاط المضاد للأكسدة (مختبريا) . تم الحصول على إجمالي الفينول والفلافونيدات بواسطة طرق Folin-Ciocalteu وكلوريد الألومنيوم على التوالي ، وتم توصيف المستخلص المائي الميثانولي الخام بواسطة HPLC UV-DAD. تم إجراء أنشطة مضادات الأكسدة في المختبر باستخدام ٢،٢-ثنائي فينيل ١-بيكريل هيدرازيل (DPPH) ، و اختبار القدرة الأرجاعية للحديد واختبارات β -carotene-linoleic acid. تفاوت إجمالي محتويات المستخلصات الفينولية والفلافونويدية بين ١٤،٥٦-٢٣٧،٦ مغ /GAE غ مستخلص و ١،٦٣ - ٢٧.٢ مغ /QE غ مستخلص ، على التوالي حيث يكون لجزء أسيتيل الأسيتات أعلى قيمة. كشف توصيف مستخلص الميثانول المائي عن وجود حمض الساليسيليك ، وحمض الكوماريك ، وحمض السينزسيك وحمض روزمارينيك ، ونارجينين ٧-جلوكوزيد ، ولوتولين ٧-جلوكوزيد ، وفانيلين. أشارت نتائج اختبارات مضادات الأكسدة إلى أنه بالنسبة للاختبار تفخيخ الجذر الحر (DPPH) ، أظهر جزء ثنائي إيثيل الأثير نشاطا مضادا للأكسدة أقوى ، وبالنسبة لاختبار القدرة الأرجاعية، كان المستخلص الميثانولي أكثر نشاطا من الأجزاء الأخرى ، وكان جزء الكلوروفورم هو الحامي الأقوى لنظام حمض الكاروتين وحمض اللينوليك. ضد أكسدة الدهون. بالنظر إلى هذه النتائج ، يمكن استخدام *Thymus fontanesii Boiss and Reut* كمصدر لمركبات جديدة مضادة للأكسدة.