

## Identification of Phenolic Compounds and Assessment of the Antioxidant and Antibacterial Properties of *Thymelaea microphylla* Coss. et Dur. from Western Algerian Sahara (Ain-Sefra Province)

Hanane Allam<sup>1,2</sup>, Malika Bennaceur<sup>1,3</sup>, Riadh Ksouri<sup>4</sup>, Rabéa Sahki<sup>5</sup>, Abderrazak Marouf<sup>6</sup>, Houari Benamar<sup>1,3,7,\*</sup>

<sup>1</sup> Department of Biology, Faculty of Natural Sciences and Life, University of Oran1, El M'Naouer, Oran, Algeria

<sup>2</sup> Department of Biology, Faculty of Natural Sciences, Life, Earth and Universe Sciences, University of Tlemcen Abou Bekr Belkaid, Tlemcen, Algeria

<sup>3</sup> Laboratory of Research in Arid Areas, Department of Biology and Physiology of Organisms, Faculty of Biological Sciences, University of Science and Technology Houari Boumediene, El Alia, Bab Ezzouar, Algiers, Algeria

<sup>4</sup> Laboratoire des Plantes Aromatiques et Médicinales, Centre de Biotechnologie de Borj-Cédria, Hammam-Lif, Tunisia

<sup>5</sup> National Institute of Forest Research, Tamanrasset, Algeria

<sup>6</sup> Department of Natural Sciences and Life, Institute of Science and Technology, University Center of Naama, Naama, Algeria

<sup>7</sup> Department of Biology, Faculty of Natural Sciences and Life, University of Mostaganem Abdelhamid Ibn Badis, Mostaganem, Site III, Algeria

### ABSTRACT

*Thymelaea microphylla* is a medicinal plant commonly used in the Algerian Sahara traditional medicine and has a chemical composition with therapeutic properties. In the current study, the antioxidant activities of the ethyl acetate, methanol, and aqueous extracts from aerial parts of *T. microphylla* were evaluated by means of phosphomolybdenum, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS),  $\beta$ -carotene bleaching, and reducing power assays. Antibacterial effect was also determined using disc diffusion method. The tested samples exhibited an important antioxidant effects by scavenging free radicals, inhibition of  $\beta$ -carotene oxidation, and by acting as reducing agents. The high antioxidant capacity was found in extracts with high phenolic content. Methanol extract with high content of phenolic compounds and flavonoids showed the best antioxidant effect. The extracts showed also antibacterial activity against human pathogenic bacteria. Methanol extract was the most active among the tested extracts against Gram-positive and Gram-negative bacteria. The high-pressure liquid chromatography (HPLC)-UV-diode array detector (DAD) analysis leads to the identification of phenolic acids, flavonoid glycosides and aglycones. The results suggest a possible application of *T. microphylla* as a potential natural source of bioactive compounds with antioxidant and antibacterial activities.

**Keywords** Aerial Part Extracts, Biological Activities, Flavonoids, HPLC-UV-DAD, Phenolic Compounds, Phytochemical Analysis.

### 1. INTRODUCTION

Thymelaeaceae is a small family comprising about 1200 species with 67 genera. The species of this family are

\* [houaribenamar@hotmail.com](mailto:houaribenamar@hotmail.com);

[houari.benamar@univ-mosta.dz](mailto:houari.benamar@univ-mosta.dz)

Received on 27/4/2019 and Accepted for Publication on 12/2/2020.

distributed in tropical and temperate zones of the earth<sup>1</sup>. The genus *Thymelaea* contains 30 species of evergreen shrubs, and eight are distributed in Algeria. *T. microphylla* Coss. et Dur. is an under shrub with dioic flowers and clusters. The leaves are very small, ovoid, scattered and distant on the branches. The stems are highly branched<sup>2</sup>. It is a rare medicinal plant endemic to Algeria, occurs in the arid and desert zones of Algeria<sup>3</sup> and is called "Methane".

This species has been used in folk medicine in Algeria for the treatment of hair loss, depression, abscess, wounds and various cutaneous conditions such as erysipelas, pimples, skin cancer, inflammations, diabetes, helminthiasis, and infections in the urinary tract<sup>4-7</sup>.

A variety of biological activities have been reported in the literature for *T. microphylla* from Algeria, including antiproliferative (aerial parts)<sup>8</sup>, hypoglycemic and anti-inflammatory activities (leaves and flowers)<sup>7,9</sup>.

Previous chemical studies on the aerial parts of *T. microphylla* have reported the presence of dihydroxylated monoterpenes and monoterpene glucosides, triterpenoids, spiro- $\gamma$ -lactone glycosides, phytosterols, phenolic acid derivatives, phenylpropanoid glucosides, simple coumarins, bis-coumarins, lignans, flavonoid glucosides, biflavonoids, ionol glucosides, benzyl alcohol glucosides, and alkaloids<sup>3,5,8,10-12</sup>.

In continuation of our ongoing research program on the exploitation of Algerian plants<sup>13,14</sup>, we describe herein the antioxidant and antibacterial activities of different extracts from aerial parts of *T. microphylla*. Also, in this study, the total phenolic, flavonoid and tannin contents, and the phenolic profile of extracts were determined.

## 2. Materials and methods

### Chemicals and reagents

*n*-hexane, ethyl acetate, ethanol, methanol, acetonitrile, chloroform, dimethyl sulfoxide (DMSO), trifluoroacetic acid (TFA), trichloroacetic acid (TCA), sulfuric acid, gallic acid, chlorogenic acid, ellagic acid, sinapic acid, syringic acid, *trans*-3-hydroxycinnamic acid, *trans*-cinnamic acid, ascorbic acid, linoleic acid, quercetin, isoquercitrin, catechin, apigenin, myricetin, kaempferol 3-*O*-rutinoside, resorcinol, vanillin,  $\beta$ -carotene, potassium persulfate, potassium ferricyanide, ferric chloride, sodium phosphate, ammonium molybdate, DPPH, ABTS, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), trolox, tween 40, hydrogen peroxide, Mueller-Hinton agar, ampicillin, oxacillin, and gentamicin were purchased from

Sigma (St. Louis, USA). Thin layer chromatography (TLC) silica gel 60 F254 aluminium plates were purchased from Merck (Darmstadt, Germany). All other chemicals, if not specified, were purchased from Sigma-Aldrich.

### Plant material

*T. microphylla* was collected in march 2015 from Ain-Sefra, Naama, Algeria. The species was identified by one of the author (AM). A voucher specimen of the plant (OUE.2015.C10) was deposited in the collections of the laboratory of the first author. The aerial parts were dried in a well-ventilated room at a temperature of 30°C and stored in the dark until use.

### Extraction

The dried powder of aerial parts (100 g) was first defatted by *n*-hexane (600 ml) at room temperature for 24 h three times, and then the residue was extracted successively with ethyl acetate and methanol in the same way. All extracts were filtered through a filter paper. The organic solvent was removed under reduced pressure at 40°C, to yield ethyl acetate and methanol extracts, respectively. For aqueous extract, dried powder of aerial parts (50 g) was extracted three times under reflux by distilled water (500 ml) for 30 min. The extracts were filtered through filter paper, combined and lyophilized to afford the aqueous extract.

### Phytochemical analysis

Phytochemical analysis was performed according to Wagner and Bladt, 1996<sup>(15)</sup> to detect the presence of different plant secondary metabolite classes.

### Identification of phenolic compounds using HPLC-UV-DAD

HPLC-UV-DAD analysis was performed on LC Agilent Technologies 1100 Infinity series (Agilent Technologies, Palo Alto, CA, USA) equipped with an auto-sampler model 1100, a quaternary pump model 1100, and diode array detector model 1100. A C18 column (250 mm  $\times$  4.0 mm, 5  $\mu$ m, Bischoff Analysentechnik GmbH, Leonberg, Germany) was used for analysis. The mobile phase was composed of two solvents; (A): 0.025% TFA in

H<sub>2</sub>O and (B): acetonitrile. The sample was prepared at concentration of 10 mg/ml in methanol/H<sub>2</sub>O (1:1) and filtered through a 0.45 µm Millipore filter (Millipore Corp., Bedford, Mass., USA). The elution program at 1 ml/min was as follows: 10-50% B (0-40 min), 50-100% B (40-41 min), 100% B (41-50 min), 100-10% B (50-55 min), 10% B (55-59 min). The injection volume was 10 µL and peaks were monitored at 280 nm. Peaks were identified by congruent retention times and UV spectra, and compared with those of the standards. The contents of the identified compounds were obtained from calibration curve with standards.

#### **Quantification of total phenolic content (TPC)**

The total phenolic content (TPC) of extracts was determined according to Singleton et al., 1999<sup>(16)</sup>.

#### **Quantification of total flavonoid content (TFC)**

The total flavonoid content (TFC) of extracts was estimated by the method described by Kim et al., 2003<sup>(17)</sup>.

#### **Quantification of total condensed tannin content (TCTC)**

Proanthocyanidins were measured using the modified vanillin assay described by Sun et al., 1998<sup>(18)</sup>. To 50 µl of suitably diluted samples, 3 ml of methanol vanillin solution (4%) and 1.5 ml of concentrated sulfuric acid were added. The mixture was allowed to stand for 15 min and the absorbance was measured at 500 nm against methanol as a blank. The amount of total condensed tannin was expressed as mg catechin equivalents (CE)/g dried extract (DE).

#### **Antioxidant activities**

##### **Determination of total antioxidant capacity (TAC)**

Total antioxidant capacity (TAC) of extracts was evaluated through the assay of a green phosphate/Mo<sup>5+</sup> complex according to the method described by Prieto et al., 1999<sup>(19)</sup>. An aliquot (0.1 ml) of diluted extracts was combined with 1 ml of reagent solution (0.3 N sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Methanol was used instead of sample for the

blank. The tubes were incubated in a boiling water bath for 90 min. Then, the samples were cooled to room temperature and the absorbance was measured at 695 nm with a UV-visible spectrophotometer (Optizen 2120 UV, Mecasys Co., Ltd, Korea) against blank. TAC was expressed as mg gallic acid equivalents (GAE)/g dried extract (DE).

##### **DPPH radical scavenging activity**

The DPPH radical scavenging activity was measured according to the procedure described by Cavin et al., 1998<sup>(20)</sup>. Methanolic solution (5 µl) of each sample at five different concentrations was added to 915 µl of methanol and then 200 µl of DPPH solution were added (0.022% in methanol). The mixture was incubated at room temperature in the dark and the absorbance of the reaction mixture was measured at 517 nm after 30 min, against a blank of methanol without DPPH. The DPPH solution without sample solution was used as control. Quercetin, ascorbic acid, and BHT were used as reference compounds. Scavenging activity was determined by the following equation (1):

$$\text{scavenging activity(\%)} = 100 \times (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \quad (1)$$

The percentage of scavenging activity was then plotted against the antioxidant concentration to obtain the amount of antioxidant necessary to decrease the initial solution of DPPH by 50% (IC<sub>50</sub>).

##### **2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation scavenging activity**

ABTS radical cation was produced by mixing 5 ml of 7 mM ABTS solution and 5 ml of 2.45 mM potassium persulfate. The mixture was stored in the dark for 16 h and diluted with ethanol to get an absorbance of 0.7 at 734 nm. The reaction medium comprised 950 µl of ABTS solution and 50 µl of each sample at various concentrations. The reaction medium was homogenized and its absorbance was recorded at 734 nm after 6 min<sup>21</sup>. BHT and trolox were used as reference compounds. ABTS scavenging ability was expressed as IC<sub>50</sub>, the inhibition percentage of ABTS

radical cation was calculated using the above formula (1).

#### **β-carotene/linoleic acid assay**

According to Kartal et al., 2007<sup>(22)</sup>, 1 mg of β-carotene was dissolved in 2 ml of chloroform. The solution of β-carotene-chloroform was introduced into a flask containing 25 μl of linoleic acid and 200 mg of Tween 40. The chloroform was completely evaporated by using a vacuum evaporator. Then, 100 ml of hydrogen peroxide were added slowly with vigorous stirring. 2.5 ml of this new solution were transferred into tubes and 350 μl of each extract were added (1 mg/ml in methanol). The test tubes were incubated in darkness at laboratory temperature. Two control tubes were also prepared with the same procedure, one containing an antioxidant reference BHT (positive control) and the other without antioxidant (negative control), where the sample was replaced by 350 μl of methanol. Absorbance was immediately measured at 490 nm. Other readings were recorded at different time intervals (2, 4, 6, 12, and 48 h). The relative antioxidant activity of the extracts (RAA) after 48 h was calculated according to the following equation:

$$RAA(\%) = (Abs_{48h \text{ sample}} / Abs_{48h \text{ BHT}}) \times 100$$

Where Abs<sub>48h sample</sub> is absorbance of sample after 48 h and Abs<sub>48h BHT</sub> is that of BHT after 48 h.

#### **Reducing power activity (RPA)**

The ability of the plant extracts to reduce Fe<sup>3+</sup> to Fe<sup>2+</sup> was assayed by the method of Oyaizu, 1986<sup>(23)</sup>. Sample solutions at different concentrations were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50 °C for 20 min. Afterwards, 2.5 ml of TCA (10%) were added and the mixture was centrifuged for 10 min at 1000 × g. Supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml of ferric chloride solution (0.1%), and the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power. The EC<sub>50</sub> value is the extract concentration at which the absorbance was 0.5 for the reducing power and was calculated from the graph of

absorbance at 700 nm against the extract concentrations. BHA and trolox were used as positive controls.

#### **Antibacterial activity**

##### **Bacterial strains**

The extracts were individually tested against a panel of six bacteria species, one Gram-positive (*Staphylococcus aureus* ATCC 25923) and five Gram-negative (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Proteus mirabilis* ATCC 12453, *Proteus vulgaris* ATCC 8427, and *Pseudomonas aeruginosa* ATCC 27853). All of the bacterial strains were obtained from the Bacteriology Laboratory of Saidalgroup, SPA, Algiers, Algeria.

##### **Disc diffusion method**

Disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method by Bauer et al., 1966<sup>(24)</sup> to assess the presence of antibacterial activity in plant extracts. A bacterial culture (which has been adjusted to 0.5 McFarland standard) was used to lawn Mueller-Hinton agar plates evenly using a sterile swab. The plates were dried and then used for the sensitivity test. The discs, which had been impregnated with a series of plant extracts (dissolved completely in DMSO at concentration of 1 mg/ml), were placed on the Mueller-Hinton agar surface. Each test plate comprised five discs; one positive control, which is a standard commercial antibiotic disc, one negative control, and three treated discs. The plates were then incubated at 37°C for 18 to 24 h depending on the species of bacteria used in the test. After the incubation, the plates were examined for inhibition zone. The inhibition zone was measured using calipers and recorded. Ampicillin (10 μg/disc), oxacillin (1 μg/disc), and gentamicin (10 μg/disc) were used as positive controls against bacteria. The negative control was DMSO.

##### **Statistical analysis**

All the tests were carried out in triplicate. Results were expressed as mean ± standard error mean (S.E.M.). Statistical analysis was performed by one-way analysis of

variance (ANOVA) followed by Tukey and Student-Newman-Keul's post hoc test for multiple comparisons. Statistical analysis was performed by using IBM SPSS statistics V24 software from IBM. A value of  $P < 0.05$  was considered to indicate statistical significance.

### 3. Results

#### Phytochemical screening

The phytochemical analysis of plant extracts showed the presence of numerous classes of plant phytochemicals, namely flavonoids, phenolic acids, tannins, coumarins, saponins, sesquiterpenes, and cardiotoxic glycosides. On the other hand, the extracts were negative to alkaloids, lignans, anthracene derivatives and anthraquinones, naphthoquinones, and free quinones (Table 1).

#### HPLC-UV-DAD analysis

The peak chromatograms of different extracts of *T. microphylla* resulting from the HPLC-UV-DAD analysis are shown in Figure 1. The amounts of the detected compounds are presented in Table 2. Thirteen phenolic compounds were identified, with kaempferol 3-*O*-rutoside (**10**) (4.849 mg/g DE) and *trans*-3-hydroxycinnamic acid (**6**) (4.744 mg/g DE) as the major compounds among the quantified phenolic compounds. Furthermore, the flavonoid glycoside kaempferol 3-*O*-rutoside (**10**) was identified in all extracts.

#### Total phenolic, flavonoid and tannin contents

The total phenolic, flavonoid, and tannin contents of extracts are shown in Table 3. High TPC were obtained in methanol and ethyl acetate extracts with values of  $317.08 \pm 0.86$  and  $218.61 \pm 0.56$  mg GAE/g DE, respectively, while the lowest content ( $184.67 \pm 0.38$  mg GAE/g DE) was recorded with the water extract.

Flavonoids were also quantified. Ethyl acetate and aqueous extracts showed low contents of flavonoids ( $89.73 \pm 0.23$  and  $66.79 \pm 0.50$  mg CE/g DE, respectively), whereas methanol extract gave a significant high content ( $172.27 \pm 0.58$  mg CE/g DE).

TCTC varied from  $33.08 \pm 0.93$  mg CE/g DE recorded

in the water extract to  $96.39 \pm 0.67$  mg CE/g DE in the ethyl acetate extract. As shown in Table 3, these amounts varied significantly with type of solvent and ethyl acetate extract showed the highest TCTC.

#### Antioxidant activities

The antioxidant activities of the different extracts from the aerial parts of *T. microphylla* were evaluated using different assays and the results are shown in the Table 4, Figure 2 A and B.

#### Determination of total antioxidant capacity

The TAC of the methanol and aqueous extracts of *T. microphylla* ( $226.40 \pm 1.15$  and  $204.76 \pm 0.76$  mg GAE/g DE, respectively) evaluated by phosphomolybdenum method was higher than that of the ethyl acetate extract ( $186.75 \pm 0.69$  mg GAE/g DE) (Table 4).

#### DPPH radical scavenging activity

The antioxidant potentials measured by the DPPH assay are shown in Table 4. The methanol extract was more efficient in the reduction of DPPH<sup>•</sup> with an IC<sub>50</sub> value of  $7.50 \pm 0.21$  μg/ml, than the ethyl acetate and aqueous extracts that gave an IC<sub>50</sub> of  $9.86 \pm 0.11$  and  $12.80 \pm 0.23$  μg/ml, respectively. The activity of all extracts was less than quercetin (IC<sub>50</sub> of  $4.67 \pm 0.03$  μg/ml), ascorbic acid (IC<sub>50</sub> of  $5.16 \pm 0.03$  μg/ml), and BHT (IC<sub>50</sub> of  $5.32 \pm 0.02$  μg/ml), used as reference antioxidants in this test.

#### ABTS radical cation scavenging activity

The capacity of the extracts from aerial parts of *T. microphylla* to scavenge free radicals was also measured by their ability to quench ABTS<sup>•+</sup>. Table 4 depicts the concentration-dependent decolorization of ABTS<sup>•+</sup>, given as IC<sub>50</sub> values. As showed in Table 4, methanol and ethyl acetate extracts showed the highest capacity with IC<sub>50</sub> values of  $15.80 \pm 0.40$  and  $17.80 \pm 0.35$  μg/ml, respectively. Water extract was found to be the weakest in ABTS radical cation scavenging (IC<sub>50</sub> of  $31.10 \pm 0.71$  μg/ml). The positive controls trolox and BHT displayed a stronger effect (IC<sub>50</sub> of  $2.43 \pm 0.03$  and  $3.55 \pm 0.02$  μg/ml, respectively).

### **β-carotene/linoleic acid bleaching activity**

The kinetics of bleaching of β-carotene in the presence of extracts of *T. microphylla*, BHT, and negative control are shown in Figure 2 A. The inhibition capacity followed the same trail than that of the methods cited above, methanol extract exhibited an interesting β-carotene bleaching inhibition after 48 h and was more efficient than the ethyl acetate and water extracts. However, standard antioxidant (BHT) showed the highest inhibition capacity at 48 h. On the one hand, it can be seen in Figure 2 B that the extract rich in phenolic compounds and flavonoids, that is, methanol extract (RAA of 80.54%) is more active than the other extracts.

### **Reducing power activity**

The results of RPA assay are shown in Table 4. Iron ion reduction capacity of the extracts was expressed as values of absorbance at 0.5; determined from absorbance curves at the wavelength of 700 nm. The RPA was found to be concentration dependent (results not shown). The results indicated that the highest activity was noted for methanolic extract of *T. microphylla* (EC<sub>50</sub> of 30.44 ± 0.58 μg/ml), followed by ethyl acetate extract (EC<sub>50</sub> of 38.25 ± 0.55 μg/ml). The lowest EC<sub>50</sub> value was recorded with aqueous extract (EC<sub>50</sub> of 43.89 ± 0.02 μg/ml). BHA and trolox used as references antioxidants showed a strong reducing power of iron ion (EC<sub>50</sub> of 3.01 ± 0.02 and 4.89 ± 0.02 μg/ml, respectively).

### **Antibacterial activity**

The three extracts of *T. microphylla* were screened for their antibacterial effects against standard strains of *S. aureus*, *E. coli*, *P. mirabilis*, *P. vulgaris*, *P. aeruginosa*, and *K. pneumoniae*. The results of the extracts are presented in Table 5. The effect of extracts was compared to the standard agents. According to the results obtained, all extracts exhibited inhibition zones against *S. aureus* with high activity for aqueous extract (diameter inhibition of 36.00 ± 0.58 mm) compared to the standard agent gentamicin (diameter inhibition of 13.00 ± 0.00 mm). There was antibacterial activity with ethyl acetate and

methanol extracts against *P. vulgaris* (diameter inhibition of 14.00 ± 0.58 and 20.67 ± 0.67 mm, respectively). The methanol extract was also active against *P. mirabilis* with diameter inhibition of 7.33 ± 0.33 mm. However, all extracts were inactive against *E. coli*, *P. aeruginosa*, and *K. pneumoniae*. No activity was recorded for standard agents against *K. pneumoniae*.

## **4. Discussion**

The results of phytochemical screening of extracts from areal parts of *T. microphylla* indicate the presence of flavonoids, phenolic acids, tannins, cardiogenic glycosides, and saponins which corroborated to the earlier report by Dehimi et al., 2016<sup>(9)</sup>. However, the extracts revealed the absence of alkaloids. Alkaloids were tested negative in extracts of *T. microphylla* as reported previously<sup>9</sup>. The identified plant secondary metabolites like flavonoids, phenolic acids, tannins, and terpenoids have been reported to have *in vitro* antioxidant and antimicrobial activities<sup>25-27</sup>.

The HPLC-UV-DAD analysis of different extracts showed the existence of six flavonoids, six phenolic acids, and one diphenol. Flavonoids like catechin (**2**), luteolin (**12**), and apigenin (**13**) have been reported to have antioxidant effect<sup>28</sup>. Phenolic acids such as chlorogenic acid (**3**) have also been implicated in antioxidant activity<sup>28</sup>. Chlorogenic acid (**3**) and *trans*-cinnamic acid (**11**) were characterized in extract of *T. hirsuta* L.<sup>29</sup>.

The phenolics were quantified in the ethyl acetate, methanol, and aqueous extracts as possible sources of bioactive substances. The results indicate significantly the effect of the solvent on the extractability of phenolics, flavonoids, and proanthocyanidins. Phenolic compounds were effectively extracted by organic solvents, methanol and ethyl acetate than the water, which gave low amount of phenolics. The results are consistent with that of Benhammou et al., 2009<sup>(30)</sup>, who obtained highest contents of phenolic compounds in methanol extract, while lowest levels were obtained with water extract. Dehimi et al., 2016<sup>(9)</sup> have obtained very low levels of

total flavonoids and proanthocyanidins in methanol extract of stem and leaf of *T. microphylla* compared to the obtained results in this study for methanol extract. Therefore, the previous reports have indicated that abiotic stresses widely present in the arid zones may be responsible for the increase of phenolics. These compounds are synthesized for a defense against oxidative stress, caused by the production of reactive oxygen species in these environmental conditions<sup>31,32</sup>. The phenolics levels of *T. microphylla* in various extracts were reported by several authors. Belyagoubi-Benhammou et al., 2014<sup>(33)</sup> have quantified total phenolic compounds in methanol extract and was estimated at 257 mg GAE/g DE. In another study, various extracts of *T. microphylla* were evaluated for amounts of phenolic compounds. The amounts were estimated in hexane, acetone, ethanol, and water extracts at 16.56; 47.59; 37.26; and 60.45 mg GAE/g DE, respectively<sup>9</sup>.

The extracts were found to show an antioxidant effect by scavenging free radicals, prevent the  $\beta$ -carotene bleaching, and act as reducing agents, which probably is due to the phenolic compounds level in all tests. Thus, methanol and ethyl acetate with significant high levels of phenolics showed high antioxidant activity. This finding is in concordance with previous studies where the strong antioxidant activity was found in plant extracts rich in phenolics<sup>34,35</sup>. Polyphenolic compounds were widely evaluated for their antioxidant effect and were found to prevent from several diseases, by their ability to counteract free radicals produced during oxidative stress. Phenolic compounds can also act as reductones, by donating the electrons and reacting with free radicals to convert them to more stable product and to terminate free radical chain reaction<sup>36</sup>. These capacities are usually associated to their structure bearing hydroxyl groups, which are able to donate a hydrogen atom<sup>37</sup>. Furthermore, despite the high polarity of methanol extract, it showed inhibition of  $\beta$ -carotene oxidation. This result can be attributed to the high amount of phenolics of the methanol extract which contribute to the

scavenging of free radicals; this test is known for its specificity to the molecules with low polarity<sup>38</sup>.

Some authors have reported the antioxidant effects of *T. microphylla* evaluated for several extracts and by numerous methods. High IC<sub>50</sub> values in DPPH test have been obtained with the water and ethanol extracts prepared from leaves and flowers of *T. microphylla* (100 and 200  $\mu$ g/ml, respectively)<sup>7</sup>. By the same method, Kerbab et al., 2015<sup>(3)</sup> have reported the IC<sub>50</sub> value of 180.80  $\mu$ g/ml for hydroalcoholic extract of areal parts. Dahamna et al., 2015<sup>(7)</sup> have also studied the antioxidant effect of water and ethanol extracts prepared from leaves and flowers evaluated by  $\beta$ -carotene bleaching test (RAA values of 46.40% and 77.86%, respectively were found). The antioxidant activity has been studied by ABTS method and the IC<sub>50</sub> values of 0.39 and 0.67  $\mu$ g/ml were obtained for water and acetone extracts of leaves and flowers, respectively<sup>9</sup>. Benhammou et al., 2009<sup>(30)</sup> have evaluated the RPA of water extract obtained from leaves and have reported the EC<sub>50</sub> value of 690  $\mu$ g/ml. Belyagoubi-Benhammou et al., 2014<sup>(33)</sup> have expressed the TAC as ascorbic acid equivalents for the methanol extract of leaf and stem and value of 14.56 mg acid ascorbic equivalents/g DE was obtained. Besides, organic extracts from other plants have been demonstrated to show also *in vitro* antioxidant activities<sup>39,40</sup>.

The results of the present work showed that, the different extracts had antibacterial activity against bacteria tested except *E. coli*, *P. aeruginosa*, and *K. pneumoniae*. In fact, *K. pneumoniae* is known to have resistance to several antibiotics<sup>41</sup>. In addition, Dahamna et al., 2015<sup>(7)</sup> have reported negative antibacterial activity against *E. coli* and *P. aeruginosa* for aqueous and ethanol extracts of leaves and flowers of *T. microphylla*. Noman et al., 2015<sup>(42)</sup> have studied the antibacterial activity of dichloromethane-methanol extract prepared from areal parts of *T. microphylla* against *S. aureus* and the inhibition diameter zone was 10.25 mm at 1 mg/ml. Aqueous extract from the areal parts of *Centaurea cyanoides* Wahlenb has been also

reported to possess antibacterial activity against *S. aureus*<sup>43</sup>.

### 5. Conclusion

The results show that among identified compounds, kaempferol 3-*O*-rutinoside (**10**) was major compound and present in all extracts. The results of antioxidant activities indicated that extracts had effective and powerful antioxidant activity, scavenging effect, reducing power, and prevent  $\beta$ -carotene bleaching in all antioxidant assays

tested. The extracts showed interesting antibacterial activity especially against *S. aureus*. For this reason, the extracts of *T. microphylla* may represent potential source of antioxidant and antibacterial agents.

### Acknowledgement

The authors would like to thank the University of Oran1 (Project PRFU number D01N01UN310120190003), LRZA, DGRSDT, Algeria, for the financial support.

**Table 1.** Secondary metabolites analysis of different extracts of *T. microphylla*.

| Secondary metabolites                  | Ethyl acetate | Methanol | Aqueous |
|--|---------------|----------|---------|
| Flavonoids                             | +++           | +++      | ++      |
| Phenolic acids                         | +++           | +++      | ++      |
| Alkaloids                              | -             | -        | -       |
| Sesquiterpenes                         | -             | +        | -       |
| Cardiotonic glycosides                 | +             | +        | -       |
| Coumarins                              | -             | +        | -       |
| Lignans                                | -             | -        | -       |
| Saponins                               | -             | +        | -       |
| Tannins                                | -             | +        | +       |
| Anthracene derivatives, anthraquinones | -             | -        | -       |
| Naphtoquinones, free quinones          | -             | -        | -       |

+ = weak; ++ = medium; +++ = strong; - = absent.

**Table 2.** HPLC-UV-DAD analysis of phenolics in different extracts of *T. microphylla*.

| Compounds   | Ethyl acetate<br>(mg/g DE) | Methanol<br>(mg/g DE) | Aqueous<br>(mg/g DE) |
|---|----------------------------|-----------------------|----------------------|
| Resorcinol ( <b>1</b> )                           | -                          | 0.252                 | -                    |
| Catechin ( <b>2</b> )                             | -                          | -                     | 2.601                |
| Chlorogenic acid ( <b>3</b> )                     | -                          | -                     | 0.528                |
| Syringic acid ( <b>4</b> )                        | -                          | -                     | 0.023                |
| Sinapic acid ( <b>5</b> )                         | -                          | -                     | 0.605                |
| <i>trans</i> -3-hydroxycinnamic acid ( <b>6</b> ) | 4.744                      | -                     | -                    |
| Isoquercitrin ( <b>7</b> )                        | -                          | -                     | 0.489                |
| Ellagic acid ( <b>8</b> )                         | 0.837                      | -                     | -                    |
| Myricetin ( <b>9</b> )                            | -                          | 0.208                 | 0.163                |
| Kaempferol 3- <i>O</i> -rutinoside ( <b>10</b> )  | 3.253                      | 4.849                 | 0.582                |
| <i>trans</i> -cinnamic acid ( <b>11</b> )         | 1.074                      | -                     | -                    |
| Luteolin ( <b>12</b> )                            | -                          | 3.959                 | -                    |
| Apigenin ( <b>13</b> )                            | -                          | 0.053                 | -                    |

Data are expressed as mg/g dried extract (DE). - = absent.

**Table 3.** Total phenolic, flavonoid, and tannin contents of different extracts of *T. microphylla*.

| Extracts      | TPC<br>(mg GAE/g DE)       | TFC<br>(mg CE/g DE)        | TCTC<br>(mg CE/g DE)      |
|---------------|----------------------------|----------------------------|---------------------------|
| Ethyl acetate | 218.61 ± 0.56 <sup>a</sup> | 89.73 ± 0.23 <sup>a</sup>  | 96.39 ± 0.67 <sup>a</sup> |
| Methanol      | 317.08 ± 0.86 <sup>b</sup> | 172.27 ± 0.58 <sup>b</sup> | 62.14 ± 0.54 <sup>b</sup> |
| Aqueous       | 184.67 ± 0.38 <sup>c</sup> | 66.79 ± 0.50 <sup>c</sup>  | 33.08 ± 0.93 <sup>c</sup> |

Values are expressed as standard error of the mean of three assays. <sup>a-c</sup>Differences within columns (samples not connected by the same letter are statistically different at  $p < 0.05$  as determined by Tukey and Student-Newman-Keul's multiple range tests). TPC: Total phenolic content, TFC: Total flavonoid content, TCTC: Total condensed tannin content. mg gallic acid equivalents/g dried extract (mg GAE/g DE), mg catechin equivalents/g dried extract (mg CE/g DE).

**Table 4.** Antioxidant activities of extracts of *T. microphylla* by different assays.

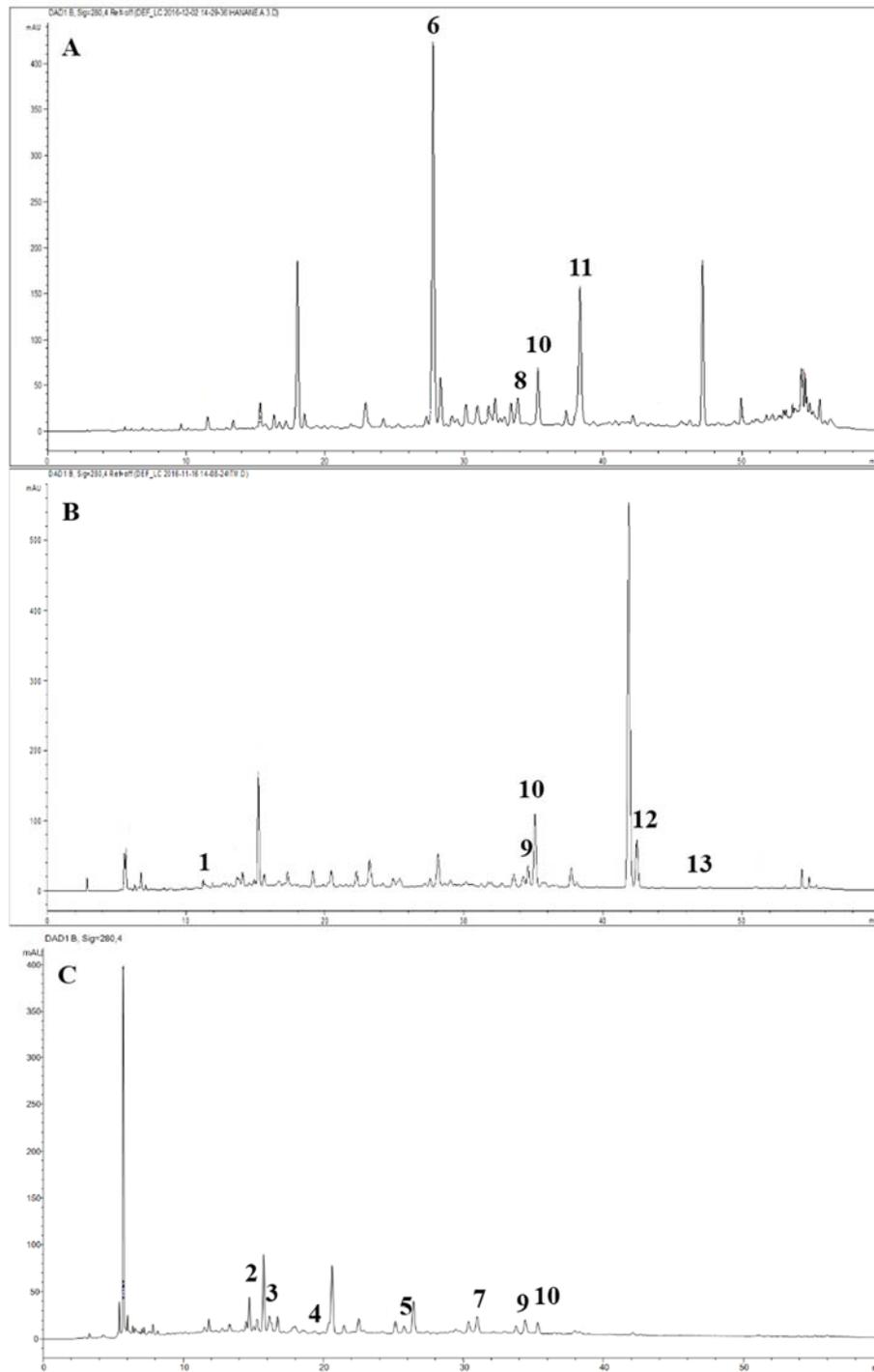
| Extracts                   | Total antioxidant capacity | 2,2-diphenyl-1-picrylhydrazyl radical | 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical | Reducing power activity   |
|----------------------------|----------------------------|---------------------------------------|--|---------------------------|
| Ethyl acetate              | 186.75 ± 0.69 <sup>a</sup> | 9.86 ± 0.11 <sup>a</sup>              | 17.80 ± 0.35 <sup>a</sup>                                      | 38.25 ± 0.55 <sup>a</sup> |
| Methanol                   | 226.40 ± 1.15 <sup>b</sup> | 7.50 ± 0.21 <sup>b</sup>              | 15.80 ± 0.40 <sup>b</sup>                                      | 30.44 ± 0.58 <sup>b</sup> |
| Aqueous                    | 204.76 ± 0.76 <sup>c</sup> | 12.80 ± 0.23 <sup>c</sup>             | 31.10 ± 0.71 <sup>c</sup>                                      | 43.89 ± 0.02 <sup>c</sup> |
| Quercetin <sup>A</sup>     | -                          | 4.67 ± 0.03 <sup>d</sup>              | n.t.   | n.t.                      |
| Ascorbic acid <sup>A</sup> | -                          | 5.16 ± 0.03 <sup>d,e</sup>            | n.t.   | n.t.                      |
| BHT <sup>A</sup>           | -                          | 5.32 ± 0.02 <sup>c</sup>              | 3.55 ± 0.02 <sup>d</sup>                                       | n.t.                      |
| BHA <sup>A</sup>           | -                          | n.t.                                  | n.t.   | 3.01 ± 0.02 <sup>d</sup>  |
| Trolox <sup>A</sup>        | -                          | n.t.                                  | 2.43 ± 0.03 <sup>d</sup>                                       | 4.89 ± 0.02 <sup>d</sup>  |

<sup>A</sup>Compounds used as positive control. Values are expressed as standard error of the mean of three assays. Total antioxidant capacity is expressed as mg gallic acid equivalents/g dried extract. <sup>D</sup>Radical scavenging activity is expressed as concentration that shows 50% activity (µg/ml). Reducing power activity is expressed as effective concentration (µg/ml) at which the absorbance is 0.5. n.t.: not tested. <sup>a-e</sup>Differences within columns (samples not connected by the same letter are statistically different at  $p < 0.05$  as determined by Tukey and Student-Newman-Keul's multiple range tests).

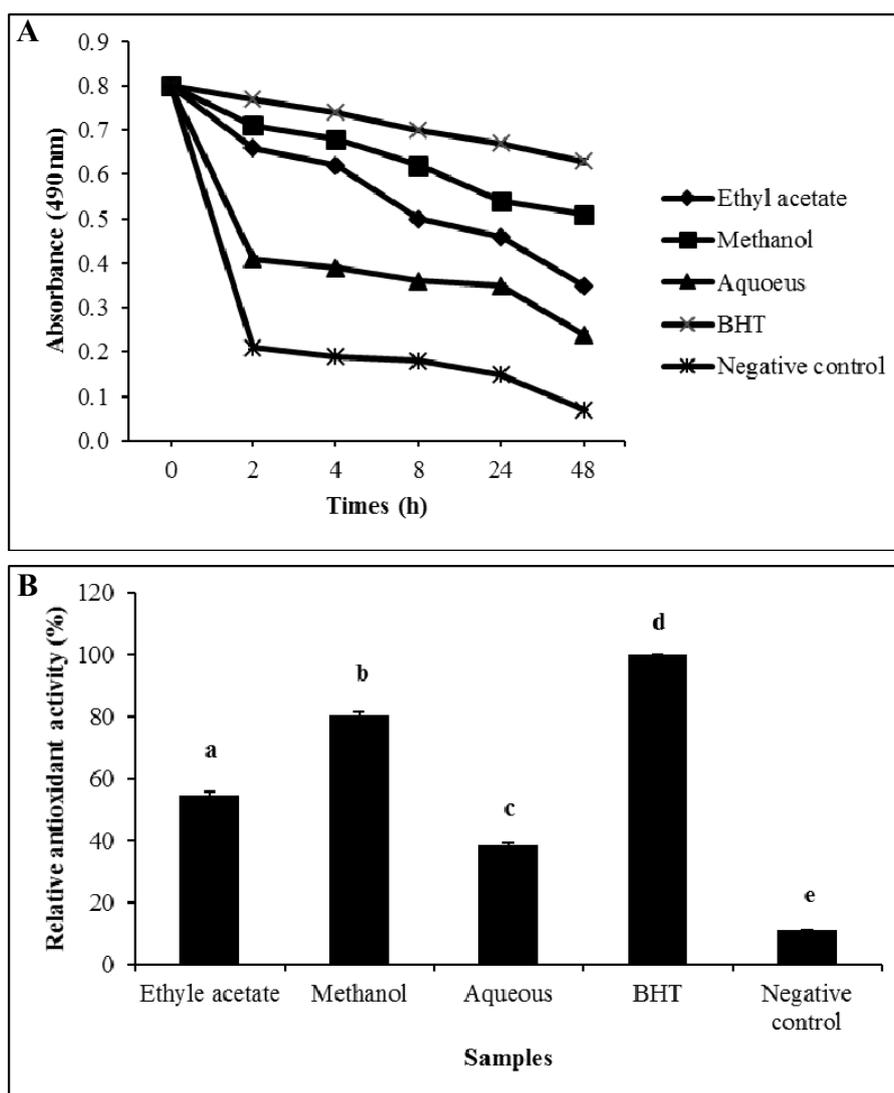
**Table 5.** Antibacterial activity of extracts of *T. microphylla* estimated by diameter of zone of inhibition (mm).

| Extracts                | <i>S. aureus</i>          | <i>E. coli</i> | <i>P. mirabilis</i>       | <i>P. vulgaris</i>        | <i>P. aeruginosa</i> |
|-------------------------|---------------------------|----------------|---------------------------|---------------------------|----------------------|
| Ethyl acetate           | 14.00 ± 0.58 <sup>a</sup> | n.a.           | n.a.                      | 14.00 ± 0.58 <sup>a</sup> | n.a.                 |
| Methanol                | 26.67 ± 0.67 <sup>b</sup> | n.a.           | 7.33 ± 0.33 <sup>a</sup>  | 20.67 ± 0.67 <sup>b</sup> | n.a.                 |
| Aqueous                 | 36.00 ± 0.58 <sup>c</sup> | n.a.           | n.a.                      | n.a.                      | n.a.                 |
| Oxacillin <sup>A</sup>  | n.a.                      | n.a.           | 29.33 ± 0.33 <sup>b</sup> | n.a.                      | n.a.                 |
| Gentamicin <sup>A</sup> | 13.00 ± 0.00 <sup>a</sup> | 8.67 ± 0.33    | n.a.                      | 24.33 ± 0.33 <sup>c</sup> | n.a.                 |
| Ampicillin <sup>A</sup> | n.a.                      | n.a.           | n.a.                      | n.a.                      | 7.33 ± 0.33          |

<sup>A</sup>Compounds used as positive control. Values are expressed as standard error of the mean of three assays. n.a.: not active. <sup>a-c</sup>Differences within columns (samples not connected by the same letter are statistically different at  $p < 0.05$  as determined by Tukey and Student-Newman-Keul's multiple range tests).



**Figure 1.** HPLC-UV-DAD chromatograms of ethyl acetate extract (A), methanol extract (B), and aqueous extract (C) of *T. microphylla* at 280 nm. Resorcinol (1), catechin (2), chlorogenic acid (3), syringic acid (4), sinapic acid (5), *trans*-3-hydroxycinnamic acid (6), isoquercitrin (7), ellagic acid (8), myricetin (9), kaempferol 3-*O*-rutinoside (10), *trans*-cinnamic acid (11), luteolin (12), apigenin (13).



**Figure 2.**  $\beta$ -carotene/linoleic acid bleaching activity. **A** - Absorbance change of  $\beta$ -carotene at 490 nm in the presence of extracts of *T. microphylla*, BHT, and the negative control. Results are expressed as the mean  $\pm$  standard error of the mean of triplicate measurements. **B** - Relative antioxidant activity of extracts of *T. microphylla*, BHT, and negative control. Results are expressed as the mean  $\pm$  standard error of the mean of triplicate measurements. Samples not connected by the same letter are statistically different at  $p < 0.05$  as determined by Tukey and Student-Newman-Keul's multiple range tests.

## REFERENCES

- (1) Borris R.P., Blasko G. and Cordell G. A. Ethnopharmacologic and phytochemical studies of the Thymelaeaceae. *J. Ethnopharmacol.* 1988; 24(1):41-91.
- (2) Quezel P. and Santa S. *Nouvelle Flore de l'Algérie et des Régions Désertiques Méridionales*; Tome II, CNRS: Paris. 1963, p 632-633.
- (3) Kerbab K., Mekhelfi T., Zaiter L., Benayache S., Benayache F., Picerno P., Mencherini T., Sansone F., Aquino R. P. and Rastrelli L. Chemical composition and antioxidant activity of a polar extract of *Thymelaea microphylla* Coss. et Dur. *Nat. Prod. Res.* 2015; 29(7):671-675.
- (4) Mouhajir F., Hudson J. B., Rejdali M. and Towers G. H. N. Multiple antiviral activities of endemic medicinal plants used by Berber peoples of Morocco. *Pharm. Biol.* 2001; 39(5):364-374.
- (5) Ghanem H., Haba H., Marcourt L., Benkhaled M. and Wolfender J. L. Microphynolides A and B, new spirogamma-lactone glycosides from *Thymelaea microphylla*. *Nat. Prod. Res.* 2014; 28(20):1732-1738.
- (6) Chermat S. and Gharzouli R. Ethnobotanical Study of Medicinal Flora in the North East of Algeria - An Empirical Knowledge in Djebel Zdim (Setif). *J. Mater. Sci. Eng.* 2015; 5(1-2):50-59.
- (7) Dahamna S., Dehimi K., Merghem M., Djarmouni M., Bouamra D., Harzallah D. and Khenouf S. Antioxidant, antibacterial and hypoglycemic activity of extracts from *Thymelaea microphylla* Coss. et Dur. *Int. J. Phytocos. Nat. Ingrid.* 2015; 2(1):15.
- (8) Noman L., Oke-Altuntas F., Zellagui A., Sahin Yaglioglu A., Demirtas I. M., Cardoso S., Akkal S., Gherraf N. and Rhouati S. A novel benzimidazole and other constituents with antiproliferative and antioxidant properties from *Thymelaea microphylla* Coss. et Dur. *Nat. Prod. Res.* 2017; 31(17):2032-2041.
- (9) Dehimi K., Speciale A., Saija A., Dahamna S., Raciti R., Cimino F. and Cristani M. Antioxidant and anti-inflammatory properties of Algerian *Thymelaea microphylla* coss. and dur. extracts. *Pharmacogn. Mag.* 2016; 12(47):203-210.
- (10) Cheriti A. and Sekkoum K. Phytochemical investigation of *Thymelaea microphylla* growing in Algeria. *Acta Chim. Slov.* 1995; 42(3):373-374.
- (11) Mekhelfi T., Kerbab K., Guella G., Zaiter L., Benayache S. and Benayache F. Phytochemical constituents of *Thymelaea microphylla* Coss. et Dur. from Algeria. *Der Pharm. Lett.* 2014; 6(1):152-156.
- (12) Noman L., Oke-Altuntas F., Zellagu A., Demirtas I. and Salah R. Isochamaejasmin and other Flavonoids Isolated from an Endemic Algerian Desert Species. *J. Turkish Chem. Soc. Sect. Chem.* 2018; 5(2):347-354.
- (13) Rached W., Benamar H., Bennaceur M. and Marouf A. Screening of the antioxidant potential of some Algerian indigenous plants. *J. Biol. Sci.* 2010; 10(4):316-324.
- (14) Benamar H., Marouf A. and Bennaceur M. Phytochemical composition, antioxidant and acetylcholinesterase inhibitory activities of aqueous extract and fractions of *Pistacia atlantica* subsp. *atlantica* from Algeria. *J. Herbs Spices Med. Plants* 2018; 24(3):229-244.
- (15) Wagner H. and Bladt S. *Plant drug analysis, a thin layer chromatography atlas*; Springer: Berlin. 1996, p 1-357
- (16) Singleton V.L., Orthofer R. and Lamuela-Raventos R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* 1999; 299:152-178.
- (17) Kim D., Chun O. K., Kim Y. J., Moon H. and Lee C. Y. Quantification of polyphenolics and their antioxidant capacity in fresh plums. *J. Agric. Food Chem.* 2003; 51(22):6509-6515.
- (18) Sun B., Richardo-da-Silvia J. M. and Spranger I. Critical factors of vanillin assay for catechins and proanthocyanidins. *J. Agri. Food Chem.* 1998; 46(10):4267-4274.
- (19) Prieto P., Pineda M. and Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphor molybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem.* 1999; 269(2):337-341.

- (20) Cavin A., Hostettmann K., Dyatmyko W. and Potterat O. Antioxidant and lipophilic constituents of *Tinospora crispa*. *Planta Med.* 1998; 64(5):393-396.
- (21) Re R., Pellegrini N., Proteggente A., Pannala A., Yang M. and Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* 1999; 26(9-10):1231-1237.
- (22) Kartal N., Sokmen M., Tepe B., Daferera D., Polissiou M. and Sokmen A. Investigation of the antioxidant properties of *Ferula orientalis* L. using a suitable extraction procedure. *Food Chem.* 2007; 100(2):584-589.
- (23) Oyaizu M. Studies on products of browning reactions: antioxidative activities of browning reaction prepared from glucosamine. *Jpn. J. Nutr. Diet.* 1986; 44(6):307-315.
- (24) Bauer A., Kirby W. W. M. M., Sherris J. C. and Turck M. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* 1966; 45(4):493-496.
- (25) Shahidi F., Janitha P. K. and Wanasundara P.D. Phenolic Antioxidants. *Crit. Rev. Food Sci. Nutr.* 1992; 32(1):67-103.
- (26) Cowan M. M. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 1999; 12(4):564-582.
- (27) Usman H., Abdulrahman F. I., Ahmed L. A., Kaita A. H. and Khan I. Z. Antibacterial effects of cyanogenic glucoside isolated from the stem bark of *Bauhinia rufescens* Lam. *I.J.B.C.S.* 2013; 7(5):2139-2150.
- (28) Rice-Evans C. A., Miller N. J. and Paganga G. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* 1997; 2(4):152-159.
- (29) Yahyaouia M., Bouajilac J., Cazaux S. and Abderrabba M. The impact of regional locality on chemical composition, anti-oxidant and biological activities of *Thymelaea hirsuta* L. extracts. *Phytomedicine* 2018; 41:13-23.
- (30) Benhammou N., Bekkara F. A. and Panovska T. K. Antioxidant activity of methanolic extracts and some bioactive compounds of *Atriplex halimus*. *C. R. Chim.* 2009; 12(12):1259-1266.
- (31) Navarro J. M., Flores P., Garrido C. and Martinez V. Changes in the contents of antioxidants compounds in pepper fruits at different ripening stages, as affected by salinity. *Food Chem.* 2006; 96(1):66-73.
- (32) Ksouri R., Megdiche W., Falleh H., Trabelsi N., Boulaaba M., Smaoui A. and Abdelly C. Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes. *C. R. Biol.* 2008; 331(11):865-873.
- (33) Belyagoubi-Benhammou N., Belyagoubi L. and Bekkara FA. Phenolic contents and antioxidant activities in vitro of some selected Algerian plants. *J. Med. Plants Res.* 2014; 8(40):1198-1207.
- (34) Djeridane A., Yousfi M., Nadjemi B., Boutassouna D., Stocker P. and Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem.* 2006; 97(4):654-660.
- (35) Hayouni E. A., Abedrabba M., Bouix M. and Hamdi M. The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. *Food Chem.* 2007; 105(3):1126-1134.
- (36) Jayaprakasha G. K., Singh R. P. and Sakariah K. K. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. *Food Chem.* 2001; 73(3):285-290.
- (37) Larson R. A. The antioxidant of higher plants. *Phytochemistry* 1988; 27(4):969-978.
- (38) Kanatt S. R., Chander R. and Sharma A. Antioxidant potential of mint (*Mentha spicata* L.) in radiation-processed lamb meat. *Food Chem.* 2007; 100(2):451-458.
- (39) Carranza M. S. S., Oyong G. G., Linis V. C., Ajero M. D. M. and Tan M. C. S. The Antioxidant and Antiproliferative Agents from the bark of Philippine *Alstoniascholaris* (L.) R. Br. (Apocynaceae). *J.J.P.S.* 2020; 13(2):207-224.
- (40) Khaled R., Lai C.-S., Pan M.-H. and Chistiane F. Study of the Antioxidant and Anti-Inflammatory Potential of the Aerial Parts of *Ficus nitida* L. (Moraceae) and its Phytochemical Composition. *J.J.P.S.* 2018; 11(2):79-83.

- (41) Nascimento G. G. F., Locatelli J., Freitas P. C. and Silva G. L. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Braz. J. Microbiol.* 2000; 31(4):247-256.
- (42) Noman L., Zellagui A., Hallis Y., Yaglioglu A. S., Demirtas I., Gherraf N. and Rhouati S. Antioxidant and antimicrobial activities of an endemic desert species *Thymelea microphylla* Coss. et Dur. *Der Pharm. Lett.* 2015; 7(1):118-121.
- (43) Shadid K. A., Al-Lahham S., Jaradat N., Abu-Nameh E. S. M. and Qaisi A. M. Preliminary Phytochemical Screening, Antioxidant and Antimicrobial Activities of the Aqueous, Methanol, Acetone, and Hexane Fractions of *Centaurea cyanoides* Wahlenb. *J.J.P.S.* 2019; 12(1): 21-32.

## تحديد المركبات الفينولية وتقييم القدرة المضادة للأكسدة والمضادة للبكتيريا لثيميليا ميكروفيليا من الصحراء الجزائرية الغربية (عين الصفراء)

- علام حنان<sup>1,2</sup>، بن ناصر مليكة<sup>1,3</sup>، رياض كسوري<sup>4</sup>، ربيعة ساحقي<sup>5</sup>، عبد الرزاق معروف<sup>6</sup>، هوارى بن عمر<sup>1,3,7</sup>
- <sup>1</sup> قسم علم الأحياء، كلية العلوم الطبيعية والحياة، جامعة وهران 1، وهران، الجزائر
- <sup>2</sup> قسم علم الأحياء، كلية العلوم الطبيعية، علوم الحياة والأرض والكون، جامعة تلمسان أبو بكر بلقايد، تلمسان، الجزائر
- <sup>3</sup> مخبر البحث على المناطق الجافة، العليا، باب الزوار، الجزائر، الجزائر
- <sup>4</sup> مخبر النباتات العطرية والطبية، مركز البيوتكنولوجيا ببرج سيدريا، حمام الأنف، تونس
- <sup>5</sup> المعهد الوطني لأبحاث الغابات، تمنراست، الجزائر
- <sup>6</sup> قسم العلوم الطبيعية والحياة، معهد العلوم والتكنولوجيا، المركز الجامعي لنعام، النعام، الجزائر
- <sup>7</sup> قسم علم الأحياء، كلية العلوم الطبيعية والحياة، جامعة مستغانم عبد الحميد ابن باديس، مستغانم، الجزائر

### ملخص

يعرف *Thymelaea microphylla* محليا باسم المثان وهو نبات طبي يشيع استخدامه في الطب التقليدي في الصحراء الجزائرية وله تركيبة كيميائية ذات خصائص علاجية. الأهداف: تهدف هذه الدراسة إلى التعرف على المركبات الفينولية وتقييم القدرة المضادة للأكسدة لمستخلصات خلات الإيثيل، الميثانول، و الماء من الأجزاء الهوائية لنبات المثان حيث استعملت أربع طرق مختلفة لتقدير النشاط المضاد للأكسدة: عن طريق فوسفوموليبدنيوم، واختبار إرجاع الجذر الحر DPPH، واختبار ABTS، وتثبيط بيروكسيدات الدهون المتمثل في اختبار تبيض البيتا كاروتين، واختبار قدرة الإختزال. كما تم تقدير النشاط المضاد للميكروبات باستخدام تقنية الانتشار من الأقراص فضلا عن تحليل المركبات الفينولية باستخدام تقنية كروماتوغرافيا السائلة عالية الأداء بمكشاف الأشعة فوق البنفسجية والمرتبطة بمطياف المجموعة الضوئية (HPLC-UV-DAD). النتائج: أظهرت العينات المختبرة قدرة عالية مضادة للأكسدة من خلال اختبار إرجاع الجذور الحرة وتثبيط أكسدة البيتا كاروتين وقدرة الإختزال. تعزى القدرة العالية المضادة للأكسدة إلى المحتوى العالي من المركبات الفينولية في المستخلصات المختبرة حيث أظهر مستخلص الميثانول الذي يحتوي على محتوى عالي من المركبات الفينولية والفلافونويدية أعلى نشاط مضاد للأكسدة كما أظهر هذا المستخلص أعلى نشاط مضاد لبعض البكتيريا المسببة للأمراض البشرية من بين المستخلصات المختبرة ضد البكتيريا الايجابية الجرام والسالبة الجرام. أسفرت تحاليل كروماتوغرافيا السائلة عالية الأداء على التعرف على بعض أحماض الفينول، جليكوسيدات الفلافونويدية وأجزائها الغير سكرية. الخلاصة: تكشف هذه الدراسة للمرة الأولى أن مستخلص الميثانول للأجزاء الهوائية لنبات المثان يتميز بالأنشطة العالية المضادة للأكسدة والمضادة للبكتيريا وغناه بالمركبات الفينولية وهو ما يدعم استخدام هذا النبات في الطب التقليدي أو استخدامه كمصدر طبيعي لمواد فعالة كمضادات الأكسدة ومضادات البكتيريا.

**الكلمات الدالة:** مستخلصات الجزء الهوائي، مضادات الأكسدة، مضادات الميكروبات، HPLC-UV-DAD، مركبات الفينول، *Thymelaea microphylla*.

تاريخ استلام البحث 2019/4/27 وتاريخ قبوله للنشر 2020/2/12.