



# Antioxidant, Antibacterial and Hypoglycemic Activity of Extracts from *Thymelaea microphylla* Coss. et Dur.

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Received 13 November 2015

Accepted 24 November 2015

ePublished 19 December 2015



## Abstract

The objective of this study was to evaluate different biological activities of aqueous and ethanolic extracts from *Thymelaea microphylla*. Antioxidant capacity was determined using two methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, and the inhibition of lipid peroxidation using the  $\beta$ -carotene/linoleate model system. Disc diffusion method was used to evaluate the antibacterial activity on four different strains. Hypoglycemic effect of aqueous extract was tested in rats. Aqueous and ethanolic extracts showed a good antioxidant activity with IC<sub>50</sub> of 0.1 and 0.2 mg/ml in DPPH test, and RRA% of 46.40% and 77.86% in  $\beta$ -carotene/linoleate assay respectively. Both extracts had no antibacterial effect on studied strains. A single administration of aqueous extract at a dose of 250 mg/kg bodyweight caused a very significant decrease in blood glucose level in rat.

**Keywords:** Antibacterial activity,  $\beta$ -carotene/linoleate, DPPH, Hypoglycemic activity, *Thymelaea microphylla*

## Introduction

*Thymelaea microphylla* Coss. et Dur., commonly known in Algeria as “Methnane” is a medicinal plant with Saharan affinity, it is a perennial with very small leaves and flowers belonging to the Mediterranean genus *Thymelaea*.<sup>1</sup> It is one of many other plants in Algerian Sahara which are insufficiently studied. According to an investigation realized with phytotherapists in the region of M'sila-Algeria, this plant is traditionally used to treat inflammations and diabetes. Biological activities of this vegetal species are studied almost for the first time in this work. Phytochemistry of *Thymelaea microphylla* is still insufficiently studied; Oleanolic acid, beta-sitosterol and 3-O-beta-D-glucopyranosyl-beta-sitosterol were detected in the aerial part.<sup>2</sup> Another study mentioned the presence of D-menthone, 2-Undecanone, Pulegone and Perillal in the essential oil of the aerial part.<sup>3</sup> Due to the use of this plant in folk medicine in the treatment of diabetes, we were interested in this study to evaluate the hypoglycemic activity of its aqueous extract and also to test its antioxidant capacity.

## Materials and Methods

### Chemicals

All the reagents used were of analytical grade (Sigma, Fluka).

### Plant Material

*Thymelaea microphylla* was collected in May 2010 from the region of Draa Elhadja in M'sila, Algeria, then leaves and flowers were separated and dried at room temperature till extraction

### Animal

Female Wistar rats (170–280 g) were purchased from Pasteur Institute in Algiers, Algeria, and rats were adapted to laboratory conditions during a month with free access to standard feed and water.

### Bacterial Strains

*Staphylococcus aureus* (G+), *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella oxytoca* (G-), were obtained from the laboratory of Microbiology, El-Zahraoui hospital (M'sila, Algeria).

### Preparation of Extracts

To prepare the aqueous extract, 50 g of plant material were macerated in 500 ml of distilled water, the mixture was heated at 70 °C for 30 min under agitation; after 3 days, it was filtered and evaporated to dryness under vacuum by using a rotavapor.<sup>4</sup> To obtain the ethanolic extract, 50 g of leaves and flowers of the



plant were macerated in 500 ml of ethanol, left for 3 days with occasional stirring, filtered and evaporated.<sup>5</sup>

#### DPPH Test

The antiradical test was performed as described by Brand-Williams et al.<sup>6</sup> 50 µl of each extract with different concentrations were added to 1250 µl of DPPH solution (0.04 mg/ml) with agitation. BHT was used as positive control and methanolic solution of DPPH as negative control. Absorbance was measured after 30 min at 517 nm and inhibition percentage of DPPH was calculated:

$$I\% = [(A \text{ blank} - A \text{ sample}) / A \text{ blank}] \times 100$$

Where A blank is the absorbance of the control reaction (containing all reagents except the test compound), A sample is the absorbance of the test compound.

#### β-Carotene/Linoleate Assay

It was carried out as described by Dapkevicius et al.<sup>7</sup> 0.5 mg of β-carotene was dissolved in 1 ml of chloroform, then 25 µl of linoleic acid and 200 mg of Tween 40 were added to the obtained solution. After evaporation of chloroform to dryness, 100 ml of oxygenated distilled water and 2.5 ml of resulted solution was added to 350 µl of each extract. BHT was used as positive control, distilled water and methanol as negative control. The absorbance was measured at 490 nm immediately, then 1, 2, 3, 4, 6, 24 and 48 h later. Relative antioxidant activity was calculated:

$$RAA\% = (A \text{ Sample} / ABHT) \times 100$$

#### Antibacterial Activity

4 different bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella oxytoca*) were used in this study. Antibacterial properties were evaluated by disc diffusion method; Bacteria were grown in nutrient broth during 6 h at 37 °C. 5 ml of 10<sup>2</sup> diluted bacterial suspensions were cultivated in Petri dishes containing Muller-Hinton medium. After 3 min, the surplus was eliminated and 6 mm discs containing extracts, Gentamicin (positive control) or DMSO (negative control) were placed on the surface of the medium. After incubation of dishes at 37 °C for 24 h, inhibition zone diameter as measured in mm.<sup>8,9</sup>

#### Hypoglycemic Activity of Aqueous Extract

3 groups of 5 rats each were formed. Animals fasted for 14 h prior to experiment; the 1st group received distilled water, the 2nd group received glibenclamid (substance with hypoglycemic effect) at a dose of 3 mg/Kg body weight and the third group received the aqueous extract of *Thymelaea microphylla* at a dose of 250 mg/Kg body weight. Blood samples were collected from tails of rats immediately before administration, then 120, 240 and 360 min later. Blood glucose levels were measured using the glucose oxidase method.<sup>10</sup>

#### Statistical Analysis

All experiments were carried out in triplicate (n=5 for hypoglycemic test). Results are expressed as mean ± S.D. Student's t-test was performed for statistical comparison.

Differences were considered significant at p<0.05. Statistical analyses were performed using the software GraphPad 5.0.

## Results and Discussion

### Antioxidant Potential

The diversity of nature and the complexity of phytochemical compounds of plant extracts impose the development of many methods to evaluate the antioxidant activity and to estimate the effectiveness of these substances. The majority of these methods are based on the coloring or the discoloration of a reagent in the reaction medium. They can be classified into two groups: those assays used in food and biological systems to evaluate lipid peroxidation while measuring the degree of oxidation inhibition (like β-carotene/linoleate method) and those assays used to measure free radical scavenging ability (like DPPH test).<sup>11</sup>

Figure 1 represents IC50 values (concentration which inhibits 50% of DPPH radical) for both extracts and some artificial antioxidants (quercetin, rutin and gallic acid) compared to BHT. Aqueous extract showed the best scavenging activity with IC50 of 0.1 mg/ml in comparison with alcoholic extract (IC50=0.2 mg/ml). The scavenging activity of the plant extracts against DPPH radical is due to the presence in their composition of active bio-molecules. According to a previous study on *Thymelaea microphylla* leaves,<sup>12</sup> aqueous extract contains 257.4 mg/g of polyphenols which are powerful chain-breaking antioxidants.

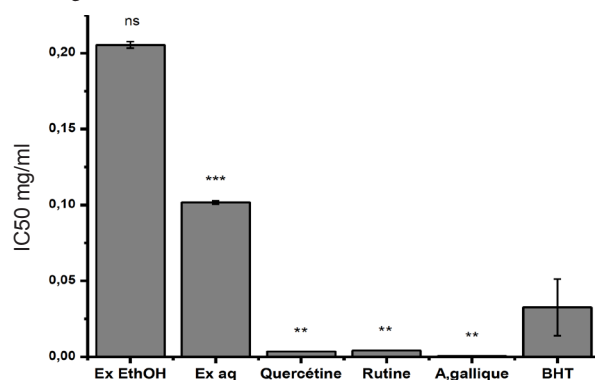
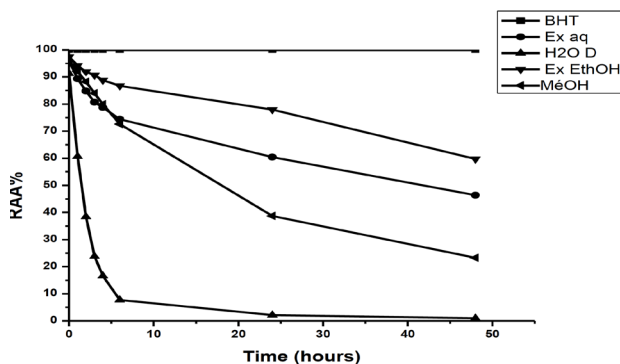


Figure 1. Values of IC50 for plant extracts and standard antioxidants (quercetin, rutin, gallic acid and butylated hydroxytoluene).

In β-carotene/linoleate assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxide formation from linoleic acid oxidation.<sup>13</sup> Figure 2 represents the variation of relative antioxidant activity (RAA%) for extracts and controls during 48 h. RAA% was determined also after 24 h for each extract (77.86% for ethanolic extract and 60.44% for aqueous extract). Unlike DPPHtest, in this one ethanolic extract was more active than aqueous extract. In general, antioxidant activities of plants' extracts is related to their richness in polyphenols present in each extract.<sup>14,15</sup>

### Disc Diffusion Method

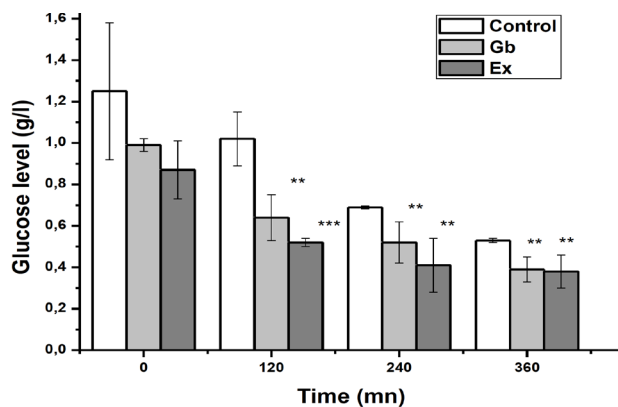
*Thymelaea microphylla* aqueous and ethanolic extracts of the leaves and flowers had a weak or no antibacterial activity against the selected bacterial strains.



**Figure 2.** Variation of Relative Antioxidant Activity during 48 h for plant extracts, BHT, methanol and distilled water.

### Hypoglycemic Activity

Glucose level was measured during 6 h for all groups, and results are presented in Figure 3. According to these results, a single dose of 250 mg/kg bodyweight administered orally to rats caused a very significant decrease in blood glucose level in comparison with control group. Glibenclamid also caused a significant decrease in glucose level; it is one of the sulfonylurea derivatives which act by increasing insulin release from the beta pancreatic cells which also stimulate the glucose transport in blood to tissues.<sup>16</sup> Other species from Thymelaeaceae family also have hypoglycemic properties like *Thymelaea hirsuta*, but no studies mentioned the chemical nature or the mechanism of action for the active compounds responsible of this effect.



**Figure 3.** Glucose levels during 6 h after oral administration of aqueous extract of *Thymelaea microphylla* Coss. et Dur.

### Conclusions

*Thymelaea microphylla* is used in folk medicine to treat diabetes type 2 and as an anti-inflammatory remedy. In this study we tried to evaluate its antioxidant and hypoglycemic activity. According to results, this plant's extracts showed a good antioxidant potential and its aqueous extract had a slight hypoglycemic effect *in vivo*. More studies are required to better know phytochemical composition of the plant and to identify the active principles responsible of these biological activities.

### Competing Interests

The authors declare no competing interests.

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