Polyphenols contents and antioxidant Activity of extracts from Leaves and flowers of *Thymelaea hirsuta*

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**ABSTRACT**

*Thymelaea hirsuta* is a medicinal plant, belonging to the genus Thymelaea (Thymelaeaceae) widely used in Mediterranean countries especially in Algeria. In this study, we have evaluated the total polyphenols and flavonoids contents of methanolic and aqueous extracts of Leaves and flowers of *Thymelaea hirsuta* as well as its antioxidant activity using the DPPH (2′,2-diphenyl-1-picrylhydrazyl) and β-carotene / linoleic acid bleaching assays. The yield of the methanolic and aqueous extract was 11, 55% and 13, 25% respectively. The total polyphenols content of the methanolic extract was 295, 22 µg GAE/mg extract and flavonoids was 09, 40 µg QE/mg and 26, 42 µg RE/mg extract. The total polyphenols content of the aqueous extract was 57, 95 µg GAE/ mg extract and flavonoids was 4.59 µg QE/ mg and 10.66 µg RE/ mg extract. In the DPPH assay, methanolic extract showed the higher scavenging capacity (IC₅₀ = 0.03 ± 0.004 mg/ml), followed by aqueous extract with IC₅₀ of 0.275 ± 0.019 mg/ml. In the test of β-carotene / linoleic acid, the percentage of inhibition was 39.1±1.33% for the aqueous extract and 41.05±2.72% for methanolic extract.

**Keywords:** Thymelaea hirsuta, antioxidant activity, polyphenols, DPPH scavenging, β-carotene.

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**INTRODUCTION**

The oxygen consumption inherent in cell growth leads to the generation of a series of reactive oxygen species (ROS). These ROS play a positive role in energy production, phagocytosis and regulation of cell growth, intercellular signalling, and synthesis of biologically important compounds. However, ROS may also be very damaging, they can attack the lipids of cell membranes and DNA. The oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases 1, 2, such as diabetes, cancer, inflammation, genotoxicity, alzheimers disease and cataracts, retinopathy, rheumatism, skin disease porphyria and senile dementia stroke 3-4.

Antioxidant is any substance that delays, prevents or removes oxidative damage to a target molecule 5. Antioxidants can scavenge free radicals and protect the human body from the oxidative stress that eventually, protect human body from cardiovascular disease, cancer, high blood pressure, diabetes and obesity 6.

Antioxidant compounds such as flavonoids, tannins, coumarins, curcumanoins, xanthons, lignans and terpenoids are found in different plant parts (e.g., fruits, leaves and seeds). Therefore, there is growing interest in separating these bioactive compounds and using them as natural antioxidants 7.

*Thymelaea hirsuta* commonly know as Methane is a shrub of the family of Thymelaeaceae. It is native from the Canary Island, Mediterranean region, north of central Europe and Eastern Central Asia 8, 9. It has been traditionally used in folk medicine as anti-septic, anti-melanogenesis 9, 10. It was shown that the aqueous extract of *Thymelaea hirsuta* possesses both hypoglycemic and antidiabetic effects in normoglycemic and streptozotocin induced diabetic rats 11, studies mentioned that the *Thymelaea hirsuta*’s aerial parts exhibited a very notable antioxidant activity 12, 13.

**MATERIALS AND METHODS**

**Plant material**

*Thymelaea hirsuta* plant material was collected from Wilaya of Sétif, Northeast of Algeria.
Preparation of plant extract

Aqueous extract

The leaves and flowers of *Thymelaea hirsuta* were washed in running water, dried and powdered. 100 g of powder was mixed with 1L of boiled distilled water (100 °C) and was placed at room temperature during 72h. The resulting mixture was filtered and then evaporated in rotary vacuum evaporator at 45°C.

Methanolic extract

The methanolic extract was obtained by maceration in water/methanol mixture (30:70) for 48h. The resultant extract was filtered through Watman paper and the solvent was removed by rotary evaporator under reduced pressure at 45°C.

Determination of total polyphenols content

Total phenolic content was determined using Folin-Ciocalteu method, according to 14 with slight modifications. A volume of 100 µL of the extract was mixed with 500 µL of Folin-Ciocalteu (diluted 10% in distilled water). After 4 min, 400 µL of sodium carbonate solution Na₂CO₃ (75 g/L) was added to the mixture, the reaction mixture was incubated at room temperature for 1h 30 min and the absorbance of the mixture was measured at 760 nm. Gallic acid (20-140 mg/L) was used as standard for the calibration curve. The total polyphenols content was expressed as micrograms of gallic acid equivalents (GAE) per milligram of extract. All samples were analyzed in three replications.

Evaluation of antioxidant activity

DPPH free radical-scavenging assay

The free radical scavenging activity of the extracts was measured by 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay 16. After dissolving the aqueous extract in distilled water, the methanol extract in methanol, the solution of DPPH in methanol (0.04mg/mL) was prepared and 1250 µL of this solution was added to 50µL of extracts solution at different concentration. The mixture was shaken vigorously and then kept in the dark for 30 minutes at room temperature. Then, the absorbance was measured at 517nm. BHT and gallic acid were used as standards. All tests were performed in triplicate. Radical-scavenging activity was calculated using the following equation:

\[
\text{Radical scavenging activity (％)} = (A_{\text{blank}-A_{\text{sample}}}/A_{\text{blank}}) \times 100
\]

Table 1: Total polyphenols and flavonoids content of *Thymelaea hirsuta* extracts.

| Extract   | Polyphenols µg GAE/mg extract | Flavonoids µg QE/mg extract | Flavonoids µg RE/mg extract |
|-----------|-------------------------------|-----------------------------|-----------------------------|
| AqE       | 57,95                         | 4,59                        | 10,66                       |
| ME        | 295,22                        | 9,40                        | 26,42                       |

AqE: aqueous extract, ME: methanolic extract, GAE: gallic acid equivalent, QE: quercetin equivalent, RE: rutin equivalent. Each value represents the mean ± SD (n = 3).
DPPH radical scavenging activity

DPPH radical is a stable free radical that shows a maximum absorption at 517 nm, and is widely used to evaluate the free radical scavenging ability of natural compounds. In the DPPH assay, the antioxidants were able to reduce the stable radical DPPH (purple color) to the yellow colored diphenylpicrylhydrazine. Therefore, the antioxidant activities of a sample can be expressed as its ability in scavenging the DPPH radical.

Results of DPPH scavenging activity of *Thymelaea hirsuta* extracts are given in Table 2. ME exhibited the highest activity toward DPPH scavenging (IC$_{50}$ = 0.030 ± 0.004 mg/ml) followed by AqE with (IC$_{50}$ = 0.275 ± 0.019 mg/ml).

### Table 2: DPPH scavenging activity of *Thymelaea hirsuta* extracts and standards.

| Extracts | IC$_{50}$(mg/mL) |
|----------|------------------|
| AqE      | 0.275 ± 0.019    |
| ME       | 0.030 ± 0.004    |
| Gallic acid | 0.001 ± 0.000*  |
| BHT      | 0.043 ± 0.003*   |

#: μg/ml. Each value represents the mean ± SD (n = 3).

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

The results of the inhibition of β-carotene oxidation in the presence of extracts after 24 hours of incubation was presented in Table 3. The antioxidant activity of the total extracts in the β-carotene/linoleic acid assay was (41.05 ± 2.72%) for the methanolic extract and (39.1 ± 1.33%) for the aqueous extract.

### Table 3: Antioxidant activities of *Thymelaea hirsuta* extracts at 24 hours of incubation measured by β-carotene bleaching method.

| Extracts | Inhibition % |
|----------|--------------|
| AqE      | 39.1 ± 1.33  |
| ME       | 41.05 ± 2.72 |
| BHT      | 100 ± 2.76   |
| H2O      | 9.162 ± 0.528|
| methanol | 33.83 ± 2.891|

Each value represents the mean ± SD (n = 3).

*Thymelaea hirsuta* extracts are rich sources of natural antioxidants which appears to be an alternative to synthetic antioxidants. The chemical composition of flower, stem and leaf of *Thymelaea hirsuta* indicated the presence of phenolic compounds including flavonoids, which are known to possess antioxidant activities.

In general the antioxidant activity of phenolic compounds reportedly varies with the structure and degree of hydroxylation of the aromatic ring. It is associated with the number of hydroxyl groups and the most active possess from 3 to 6 hydroxyl groups. Hydroxylation in the C3 position seems to be detrimental for their antioxidant potency. Fukumoto and Mazza reported that for benzoic and cinnamic acid derivatives, flavonols and anthocyanidins, an increase in the number of hydroxyl groups on the aromatic ring lead to higher antioxidant activity in vitro. Compounds with three hydroxyl groups on the phenyl ring of phenolic acids or the B-ring of flavonoids had high antioxidant activity. The loss of one hydroxyl group decreased activity slightly. Moreover, screening of phytochemical compounds in *Thymelaea hirsuta* revealed the presence of tannins, alkaloids, steroids, saponins, coumarins, reductores compound and anthraquinones. The antioxidant activities of *Thymelaea hirsuta* extract are due to the presence of these phytochemicals.

**CONCLUSION**

The present study aimed to evaluate the in vitro Antioxidant activity of extracts prepared from the leaves and flowers of *Thymelaea hirsuta*. The results showed that The extracts exhibited antiradical activities toward 2,2'- diphenyl-1-picrylhydrazyl (DPPH) and inhibiting lipid peroxidation.

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