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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-diphenylpicrylhydrazyl) 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 (IC_{50} = 0.03 ± 0.004 mg/ml), followed by aqueous extract with IC_{50} 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, curcumanoids, 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 Methnane 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 antiseptic, antimelanogenesis [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. 100g 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 Wattman 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–Ciocalteau (diluted 10% in distilled water). After 4 min, 400 µl of sodium carbonate solution Na_2CO_3 (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.

Determination of total flavonoids content

The total flavonoids in plant extracts were determined using the aluminum trichloride ($AlCl_3$) method [15]. Briefly, 1 ml of 2% $AlCl_3$ in methanol was mixed with 1 ml of the extract. After incubation in dark at room temperature for 10 min, the absorbance of the reaction mixture was measured at 430 nm. Quercetin (1-40 mg/l) was used as standard for calibration curve and the total Flavonoids content was expressed as micrograms quercetin equivalent (QE) per milligram of extract.

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:

$$\text{radical scavenging activity (\%)} = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

A_{blank}: Absorbance of the control.
A_{sample}: Absorbance of the reagent with extract.

β-carotene/linoleic acid assay

In this test, the antioxidant capacity of the extracts was determined by measuring the inhibition of the oxidative degradation of β-carotene (discoloration or bleaching) by the oxidation products of the acid linoleic [17]. The β-carotene solution was prepared by dissolving 0.5 mg β-carotene in 1 mL of chloroform. One milliliter of this solution was pipetted to a flask covered with aluminum foil. Then 25 µL of linoleic acid and 200 mg of tween 40 were added. The chloroform was evaporated using evaporator at 45°C. Then 100 mL of distilled water saturated with oxygen was added. 2.5 mL of this prepared β-carotene solution were transferred to test tubes, and 350 µL of the extracts (2mg/mL methanol) were added before incubation for 48h at room temperature. The same procedure was repeated with butylated hydroxyl toluene (BHT) as a positive control and with methanol and distilled water as a negative control. The absorbance was reading at 490 nm after 1h, 2h, 6h, 24h and 48h. The antioxidant activity of extracts was calculated using the following equation:

$$AA\% = A_{sample} / A_{BHT} \times 100.$$

A_{sample}: Absorbance in the presence of the extract;
A_{BHT}: Absorbance in the presence of positive control BHT.

Statistical Analyses

The results are expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) followed by the Tukey test was performed to assess differences between groups. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The yields of aqueous and methanolic extract of leaves and flowers of *Thymelaea hirsuta* were 13.25% and 11.55% respectively.

Total phenolic and flavonoid content

Plant polyphenols are secondary metabolites characterized by one or more hydroxyl groups binding to one or more aromatic rings. Several thousand polyphenolic molecules have been identified in higher plants, including edible ones. Plant polyphenols are divided into two major groups, flavonoids and non-flavonoids. Flavonoids can be divided into flavanols, flavonols, anthocyanidins, flavones, flavanones, and chalcones. Non-flavonoids include stilbene, phenolic acids, saponin, and tannins. Among the important biological properties exhibited by plant polyphenols, their antioxidant activity has raised a great interest [18].

Table 1 showed the total content of phenolics and flavonoids in extracts. AqE extract had a total phenolic content of (57,95 µg GAE/mg extract) and flavonoids (4,59 µg QE/mg extract), (10,66 µg RE/mg extract). while, Methanolic extract had a total phenolic content of (295,22 µg GAE/mg extract) and flavonoids (9,40 ± 0,06µg QE/mg extract), (26,42 µg QE/mg extract).

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

Extract	Polyphenols	Flavonoids	
	µg GAE/mg extract	µg QE/mg extract	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 coloured diphenylpicrylhydrazine. Therefore, the antioxidant activities of a sample can be expressed as its ability in scavenging the DPPH radical [19].

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 tow 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 [20]. In general the antioxidant activity of phenolic compounds reportedly varies with the structure and degree of hydroxylation of the aromatic ring [21, 22]. 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 [23]. Fukumoto and Mazza [24] 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, reducteurs compound and anthraquinones. The antioxidant activities of *Thymelaea hirsuta* extract are due to the presence of these phytochemicals [20].

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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