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EVALUATION OF PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITIES OF *ORIGANUM, CISTUS AND THYMUS* SPECIES

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ABSTRACT
The aim of this study was to evaluate antioxidant properties of four plants from North of Morocco then to quantify phenolic compounds and flavonoids in each plant extracts. The air-dried leaves of *Origanum elongatum, Cistus salvifolius, Thymus willdenowii, Boiss and Cistus laurifolius* were extracted with methanol and ethyl acetate by ultrasonic apparatus. The antioxidant effect of plant extracts was assessed using a modified 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and the quantification of phenolic compounds and flavonoids was performed by Folin Ciocalteu and alumunium trichloride reagents, respectively. The antioxidant effect of all samples, measured by calculating IC_{50} for the DDPH method, ranged from 0.27 µg/ml to 1.7 µg/ml whereas IC50 of the reference BHT was 0.67 mg/ml. The total phenolic content, analyzed using Folin–Ciocalteu's reagent, of the samples varied from 46.6 µg/100 mg to 153 µg/100 mg dry weight, expressed as gallic acid equivalents (GAE). The total flavonoid concentrations, detected using 2% aluminum chloride, varied from 3.63 to 5.54 µg equivalents (RE)/mg dry weight. The results obtained showed that the antioxidant activity of plant extracts could be correlated to the total phenolic content and the total flavonoids. In addition, *Origanum elongatum* that showed highest antioxidant activity had the highest phenolic and flavonoids contents. The evaluation of the antioxidant by the DPPH test revealed that all tested extracts have antioxidant properties and that methanol extracts are the most active. *Origanum elongatum* methanolic extract showed the best scavenging activity (IC50 = 0.267 ± 0.06 µg / ml).

KEYWORDS: *Origanum elongatum, Cistus salvifolius, Thymus willdenowii, Boiss and Cistus laurifolius.*

I. INTRODUCTION
Oxidative stress is one of the major causative factors inducing of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, aging, diabetes metillus, cancer, immunosupression, neurodegenerative diseases and others.[1] Moreover, the reactive oxygen species (ROS) can cause lipids peroxidation in food during manufacturing and storage which consequently leads to the loss of the food quality and safety.[2]

It is well known that the most effective path to eliminate and decrease the action of ROS is using antioxidants products which possess free radical chain reaction breaking properties. However, the most frequently used synthetic antioxidant such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been suspected to cause side effects on human health.[3] So there is an impetus for researches for antioxidant agents to use as alternative for food conservation and human remedies.

In recent years, much attention has been devoted to natural antioxidant and their association with health benefit. A large number of medicinal plants and their purified constituents have been reported to exhibit antioxidant activity.[4,5]

It is well known that the North of Morocco is rich of medicinal plants and some of them are endemic, present important charges of phenolic and flavonoid contents and possess significant antibacterial and antioxidant effects.[6-9]

With respect to this, our work aimed to evaluate the total phenolic content and compare the antioxidant activity of various solvent extracts from the aerial parts of five selected plants from Al-Hoceima province in the North of Morocco.

II.MATERIALS AND METHODS

II.1Plant material

The plants were collected from the North of Morocco (province of Alhoceima in the Rif mountains), and authenticated in ex-the National Institute of the Medicinal and Aromatic Plants. The plants were dried in hot air at 40 ◦C for 48 h, and then the leaves were separated from the rest of the remainder of the sample and then ground into fine powder. They were stored in the dark at 4 °C until further analysis.

II.2 Ultrasound-assisted extraction (UAE)

Extraction was carried out in an ultrasonic device (CY-500 sonicator, JP Selecta S.A. Spain) at 500 W and at a frequency of 20 Hz.[10] The dried powder sample was extracted in a 250 mL beaker at room temperature. Ultrasonic probe was directly inserted into the beaker about 4 cm under the surface of the mixture to provide direct contact with the sample for 60 min. At the end of sonication, the suspension was cooled to room temperature and then filtered through filter paper (Whatman no. 4) to remove solid debris. The solvent was removed by a rotary evaporator at temperature not higher than 40°C. The prepared extract was kept in the dark at 4°C until further.

II.2. Dosage of total polyphenols

Total polyphenols content was estimated using Folin-Ciocalteu (FC) assay which is widely used in routine analysis.[11] Briefly, all samples and gallic acid were dissolved in 50% (v/v) aqueous methanol. Samples (0.5 mL) were placed into test tubes and then 2.5 mL Folin–Ciocaltaeu reagent (10%, v/v, in water) solution and 7.5 mL sodium carbonate (20%, w/v, in water) solution were added. The tube contents were mixed and allowed to stand for 90 min at room temperature. Absorbance was measured at 750 nm and the total phenolic content was expressed as gallic acid equivalents (GAE) in mg per g of extract.

II.3. Determination of total flavonoids

The total flavonoid content was determined using the method as adapted by Arvouet-Grand and al.[12] 1.0 ml of 2% aluminum trichloride ($AlCl_3$, $6H_2O$) in methanol was mixed with the same volume of the extract solution. Absorption readings at 430 nm using Perkin Elmer UV-VIS spectrophotometer were taken after 10 min against a blank sample consisting of extract solution with 1.0 ml methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with Quercetin and expressed as mg of Quercetin equivalents per gram of sample.

II.4. Antioxidant activity

The antioxidant activity of plant extracts was measured in terms of hydrogen donating or radical scavenging ability, using the DPPH method[13-15] with some modifications.[16] Briefly, 2.5 ml of each extract and 2,5 ml of methanolic solution of DPPH were introduced in tubes. The reaction mixture was mixed thoroughly and left in the dark at room temperature for 30 min. The decrease in absorbance at 517 nm was determined with a spectrophotometer. BHT was used as a positive control and the ability of sample to scavenge DPPH radical was calculated by the following equation:

% Anti-radical activity =[(Abs control 517–Abs sample 517)/Abs control 517]x 100

Where $A_{control}$ is the control absorbance and A_{sample} is the absorbance of the extract or standard.

The radical scavenging activity (RSA) was defined by inhibition concentration (IC_{50}), which is the concentration of extract necessary to decrease the initial DPPH concentration by 50%. The lower IC_{50} value indicates higher radical scavenging capacity and vice versa.

III. RESULTS AND DISCUSSION

III.1 Total polyphenols and flavonoids

The result of total polyphenols and flavonoids contents of the four crude extracts is given in (Table 1). The determination of the polyphenols was carried out as a function of a linear calibration curve (y = ax + b) of a gallic acid solution at different concentrations (0 to 100 g / 100 ml). The flavonoid content of each extract is expressed in milligrams equivalent to Quercitine per gram of extract (mg EQ / g of extract).[17]

Table 1: Polyphenols and Flavonoids Quantities in each extract of plants.

Plant Material	Extracts	Phenols mg (EAG)/g	Flavonoïdes mg (EQ)/g
Origanum elongatum	-Ethyl acetate -Methanol	130 ± 3,05 153,22 ± 2,67	5±0,89 8,16±0,71
Thymus willdenowii.Boiss	- Ethyl acetate -Methanol	51,8 ± 0,73 56,45 ± 1,23	5,11± 0,15 5,02 ± 0,81
Cistus laurifolius	- Ethyl acetate -Methanol	46,63 ± 0,38 64,54 ± 0,33	5,02 ± 0,26 5,54± 0,9
Cistus salviifolius	- Ethyl acetate -Méthanol	80 ± 1,05 98,84 ± 0,99	4,83± 0,72 5,39±0,68

Total phenolic (TPC) and flavonoids contents (TFC) in plant extracts increase with solvent polarity. Indeed, methanolic extracts were found richer in TPC and TFC than ethyl acetate extracts (table2), especially *Origanum elongatum* which presented 153,22 ± 2,67 in mehtanolic extract against 130 ± 3,05 mg GAE / g dry in ethyl acetate one's. This observation has been proved for others species of *Origanum majorana* by Kamble et al.[18]

The TPC of extracts was in the order of *Origanum elongatum* > *Cistus salviifolius* > *Cistus laurifolius* > *Thymus willdenowii.Boiss*. A higher polarity solvent like methanol permits to extract more phenolic compounds.[19]

A similar tendency was observed for the TFC, the lowest amount was recorded in ethyl acetate extracts (4,83±0,72 to 5±0, 89 mgEQ/ g dry weight) against methanolic extract (5,02 ± 0.26 to 8,16±0,71 mgEQ/ g dry weight). The highest TFC were found in the leaves of *Origanum elongatum*, followed by *Cistus salviifolius*, *Cistus laurifolius* and t*hymus willdenowii.Boiss*.

In this context, other authors exhibited that the TPC and TFC are predominated with variable quantity in the same species, Rebaya et al proved *that the TPC* ranging from 11.96 ± 0.14 to 56.03 ± 0.06 g GAE. 100 g -1 in leaves *C.* salviifolius.[20] Our results were consistent with these values.

III. 2 Antioxidant activity

The presence of the activity is based on the discoloration of the DPPH at the level of the deposits. The more discolored the deposition is, the greater the antioxidant activity is, compared to the control The DPPH free radical-scavenging activity of the crude extract was evaluated using the DPPH method, and examined by comparing it to the activity of positive controls BHT. The concentration of sample required to scavenge 50% of DPPH (IC_{50}) was determined. The antioxidant activity of the sample is highest at lower IC_{50} value.

Table 2: antioxidant activities of methanol extracts.

Concentration µg/ml	Plante2:Origanum Elongatum	Plante3 :Cistus salviifolius	Plant4:Thymus willdenowii.Boiss	Plant5:Cistus laurifolius	BHT
200	98%	98%	98%	98%	61%
100	96%	94%	95%	95%	53,18%
50	88%	86%	67%	70%	49,87%
25	61%	80%	56%	59%	29,77%
12.5	60%	69%	50%	55%	24,17%
6.25	52,30%	59%	46%	53%	15,01%

As showed in table 2, the antioxidant activity increases with an increase in extract concentration in appreciably identical proportions that the standard. Furthermore, the extract of *O. elangatum* was found to possess the highest antioxidant activity more than BHT (88% at 50 µg/ml), Followed by *C. salviifolius, C. laurifolius and T. willdenowii.Boiss* (respectively 86%, 70% and 67% at 50 µg/ml).

The results reveal that the four extracts reduce concentration of DPPH, and that the inhibition percentage increases when concentration is intensified. We also note that all of extracts were more active (IC_{50} = 0.267 ; 0.319 ; 0.49 µg/ml) than the reference (IC50 of BHT = 0.67 µg/ml) except *C. laurifolius* which had IC_{50} (1. 672 µg/ml) higher than BHT (Figure1).

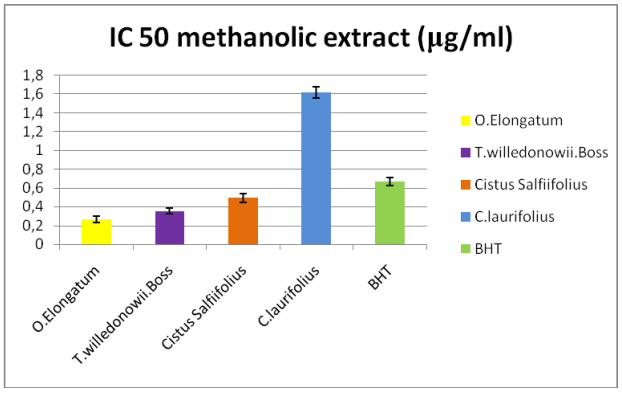

Figure 1: IC50 of methanolic extracts of the plants studied.

O. elongatum extract exhibits the highest antioxidant activity. This finding was proving by Oualili et al.[21] In fact, the authors found that *O. elongatum* had higher antioxidant activity than the *Cupressus atlantica*. The results obtained with *Origanum elongatum* were found with other *Origanum* species such as *Origanum majorana*, *Origanum compactum*, *Origanum glandulosum*, *Origanum vulgare* subsp.[21]

Published review articles demonstrated a good correlation between the total phenolic content and the antioxidant activity of analyzed plant extracts. In this

study we related the power antioxidant of our plants extracts to the polyphenols and flavonoids contents.[21, 22,23]

IV. CONCLUSION

The total phenolic content of methanolic extracts of the four plants from North of Morocco in decreasing order is *O. elongatum* > *T. willdenoii. Boiss* > *C. salviifolius* > *C.* laurifolius.

We demonstrated that antioxidant potential of all extracts is correlated with both total phenolic and flavonoids contents. This finding suggests that phenolic compounds are the major contributors to the antioxidant activity of selected plants.

The promising results obtained with *Origunum elongatum, Tymus willedenoii. Boiss and Cistus salviifolius* incite to make more profound studies.

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