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Research Article

Phytochemical Screening and Antioxidant Activity of: *Origanum elongatum* and *Cupressus atlantica* Two Endemic Plants of Morocco.

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ABSTRACT

Medicinal plants are often used for their therapeutic properties (antioxidant, antibacterial, antifungal, etc.). The objective of this work is to demonstrate the antioxidant potential and the phytochemical screening of the two endemic plants of Morocco: *Origanum elongatum* Emb and Maire and *Cupressus atlantica* Guaussen. Measures of antioxidant activity of the essential oils and the organic extracts of plants is obtained by trapping the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) in two steps: first on a silica plate and then by spectrophotometry. The results show that the essential oil and the organic extract of the two plants exhibit an antioxidant activity with an important degradation of DPPH in the organic extract of *Origanum elongatum* and *Cupressus atlantica* with a percentage of 92.3% and 90.9% respectively. Phytochemical screening reveals the presence of quinones, flavonoids, sterols and gallic tannins in *Origanum elongatum*, the presence of catechic tannis and sterols in *Cupressus atlantica* and the absence of alkaloids and saponins in both plants.

Keywords: *Origanum elongatum*, *Cupressus atlantica*, phytochemical screening, antioxidant activity.

INTRODUCTION

Medicinal plants are often used in traditional phytotherapy to treat many human pathologies like cardiovascular diseases, diabetes, cancer, hypertension, Alzheimer's disease, and so on and so forth. Oxidative stress contributes directly to these pathologies, and to find remedies, several researchers are interested in the related research in new natural antioxidants.

The geographical situation of Morocco provides a flourishing ground to various Mediterranean bioclimates which favor a biodiversity of medicinal plants with a very marked endemism[1]. In Morocco, the genus Origanum (F / lamiaceae) is represented by five species three of which are endemic including the *Origanum elongatum*. Origanum leaves are widely used by the population as culinary spices or for the treatment of dysentery, colitis, gastrointestinal disorders, bronchopulmonary disorders and mouth disorders (canker sores, gingivitis)[2]. Essential oils of origanum can act as anti-oxidants and antibacterial agents[3], anticancer and anti-inflammatory agents[4] and antifungal agents[5].

The genus Cupressus (F / Cupressaceae) includes 25 species. Atlas cypress (*Cupressus atlantica*) is an endemic specie from Morocco covering 3,000 to 5,000 ha[6]. Cupressus leaves are used to cure cough, chest infections and antidiarrheal and anti-haemorrhagic diseases[2]. The main objective of this work is to carry out a screening of the antioxidant activity of the various extracts (essential oils and organic extracts) of *Origanum elongatum* Emb and Maire and *Cupressus atlantica* Guaussen; two endemic species of Morocco hardly studied but widely used by Moroccans for therapeutic and cosmetic purposes. The antioxidant activity is studied by the DPPH method. In parallel, a phytochemical screening of the different metabolites of the two plants is carried out.

MATERIALS AND METHODS

Harvesting and pre-processing

The studied species consist of leaves, flowers of *Origanum elongatum* and *Cupressus atlantica* leaves.

Origanum elongatum Emb and Maire

The species *Origanum elongatum* was harvested in June 2013 in "Targuist" in the Rif in the north of Morocco (34 ° 57'Nord 4 ° 18'West), a mountainous region of 994 m of altitude. The leaves and flowers are dried in the shade in a dry and airy place.

Cupressus atlantica Guaussen

The species *Cupressus atlantica* was harvested in April 2013 in Beni Mellal in the Middle Atlas of Morocco (32 ° 21'Nord 6 ° 21'West), a mountainous region of 670 m of altitude. The leaves were dried in the shade in a dry and airy place.

Preparation of extracts

Essential oils

The essential oils were obtained by hydro distillation: 250 g of dried flowering leaves and summits of *Origanum elongatum* or fresh leaves of *Cupressus atlantica* were extracted using a Clevenger for 4 hours. The essential oils obtained were dried with anhydrous sodium sulfate and

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Table 1: Water content and moisture content of the two studied plants.

	Origanum elongatum	*Cupressus atlantica*
Water content (%)	59	50
Humidity level (%)	17,2	19,9

Table 2: Yield of extractions of the two studied plants (*Origanum elongatum* and *Cupressus atlantica).*

	Extraction	*Origanum elongatum*	*Cupressus atlantica*
Extract Yield	Hydrodistillation	2,8	0,7
	Solvent extraction	15,6	9,6

Table 3: Evaluation of the metabolite content of the two studied plants.

		Origanum elongatum	*Cupressus atlantica*
Free Quinones		+	-
Flavonoids		+	-
Sterols		+	+
Saponins		-	-
Alkaloids	Dragendorff	-	-
	Mayer	-	-
Tanins	Gallic	+	-
	catechin	-	+

+ Presence, - Absence

stored in the dark at 4 ° C.

Organic extracts

The powder of *Origanum elongatum* (leaves and flowers) or *Cupressus atlantica* (leaves) was extracted with a mixture of methanol / chloroform (2/1; v / v) and with constant stirring. The extracts were evaporated by a rotary evaporator and then reduced to powder by lyophilization.

Physico-chemical parameters

In order to characterize the plant material, a physicochemical screening was carried out.

Water content

The vegetal material was placed in a petri dish and then weighed (Mf). A second weighing was carried out after drying in an oven at 110 ° C. for 24 h (Ms). The water content was calculated in terms of the two measures.

Humidity

The material was mechanically crushed. The obtained powder was placed in Petri dishes with a predetermined calibrated neck. The petri dishes containing the powder were weighed and the whole was placed in an oven at 102 ° C for 24 hours. The dishes were removed from the oven and cooled for 1 hour in a desiccator before being weighed. This operation was repeated several times until the weighing was stable.

Phyto-chemical screening

The phytochemical screening of the two studied plants consisted of looking for some metabolites known for their biological activities. The tests were based on colored reactions inspired by the work of Harborne (1998)[7].

Alkaloids

The search for alkaloids was carried out by two tests: the test of Dragendorff and that of Mayer. These tests are based on the ability of the alkaloids to combine with heavy metals or with iodine [8].

Tannins

1.5 g of dry matter was solubilized in 10 ml of 80% methanol. After 15 minutes of mechanical stirring, the extracts were filtered and placed in tubes. The addition of 1% FeCl 3 was used to detect the presence or absence of tannins. The color turns to dark blue in the presence of tannins and greenish brown in the presence of catechic tannins[9].

Sterols

The presence of sterols is checked based on the reaction of Liberman-Burchard [9-10]. Three gram of dry matter was macerated in 15 ml of chloroform. After 20 minutes, the mixture was filtered and concentrated to 2 ml. 1 ml of acetic anhydride and 1 ml of concentrated sulfuric acid were successively added. The presence of sterol compounds gives a red-brown color.

Saponins

Two grams of crushed dry plant material were used to prepare a decoction with 100 ml of water for 30 min. After cooling and filtration, the volume was adjusted to 100 ml. From this stock / base solution, 10 tubes were prepared with 1,2, ... 10 ml. The final volume was readjusted to 10 ml with distilled water. Each of the tubes was agitated with energy in a horizontal position for 15 seconds. After standing for 15 minutes in a vertical position, the height of the persistent foam was measured (cm). The foam index was calculated by the following formula:

I = foam height (cm) in the 9th tube x 10 / 0.09.

The presence of saponins in the plant was confirmed with an index higher than 100.[8]

Free Quinones

1 g of powder was extracted with 15 ml of petroleum ether for 24 hours under gentle stirring. The whole was filtered and then concentrated by means of a rotary evaporator. Some drops of NaOH (1/10) were added. In the presence of free quinones, the color turned red-yellow or violet[11].

Flavonoids

1 g of the powder of each plant was extracted with 10 ml of hot distilled water for 20 min. The whole was filtered and evaporated to obtain 2 ml of extract. 2 ml of hydrochloric alcohol was added to the extract with a few magnesium turnings. The appearance of a red-orange color indicated the presence of flavonoids[8].

Screening of antioxidant activity by DPPH

The antioxidant activity of the extracts was investigated using DPPH (2,2-diphenyl-1-picrylhydrazyl). It is a stable free radical, soluble in methanol and of violet color which turns pale yellow when reduced. Two techniques were used: a screening on plate of silica based on the work of Takao et al. (1994)[12] and a spectrophotometric screening based on the work of Brand-Williams et al., (1995)[13].

Screening of antioxidant activity on silica plate

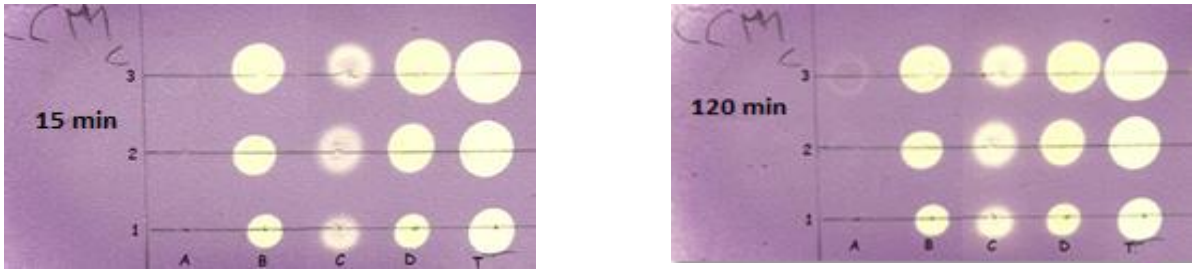

Figure 1: Image of the silica plate showing the different extracts of *Origanum elongatum* and *Cupressus atlantica* after 15 and 120 min of reaction with the DPPH at 6. 10 -5 M. *Cupressus atlantica*: A: Essential oil B: Organic extract. *Origanum elongatum*: C: Essential oil D: Organic extract. T: δ-Tocopherol at 0.4 mg / ml. Volumes deposited 1: 2 µl, 2: 4 µl and 3: 8 µl

The various extracts (essential oil and organic extract) of *Origanum elongatum* and *Cupressus atlantica* were solubilized in methanol (5 mg / ml). 3 deposits of each extract (2, 4 and 8 µl) were deposited on a silica gel plate (CCM Sil G25 UV 254 mm-Marcherey-Nagel, 5 × 20 cm, ep 0.25 mm).

Screening for antioxidant activity was carried out in comparison with reference controls: 0.01 M ascorbic acid and 0.02 M δ-tocopherol (Sigma aldriche).

The revelation of the plate was carried out by a methanolic solution of DPPH at 6.10-5M which was uniformly vaporized over the whole plate. This operation was carried out shielded from the light in order to avoid any degradation of the DPPH.

After disclosure, the plates were read at predetermined time intervals: 15 min, 30 min, 60 min, 120 min, 240 min and images of the plates were taken by scanning. The antioxidant activity of the extracts was estimated by the intensity of the discoloration of the deposits of the extracts relative to those of the controls.

Screening of antioxidant activity by spectrophotometry

The different extracts / essences from the two plants were solubilized in methanol (5 mg / ml) and 0.1 ml of the solution was mixed with 3.9 ml of methanolic DPPH (6.10 -5 M) previously prepared. The mixture was stirred and then the absorbance was measured at 517 nm at regular time intervals: 0 min, 3 min, 5 min and every 15 min up to 160 min. The purple DPPH turned yellow in the presence of antioxidants. The results were compared with the positive control which is δ-tocopherol.

RESULTS

Physico-chemical parameters and extract yields

Two parameters were measured for both plants: the water content and the moisture content are shown in Table 1.

Origanum elongatum has a moisture content of about 59%, whereas *Cupressus atlantica* has a water content of 50%. The moisture content varies according to the type of plant (Tab.1). In *Origanum elongatum*, the moisture content is about 17.2%, whereas for *Cupressus atlantica* it is 19.9%.

The yield of the various extractions (hydrodistillation and solvent extraction) is shown in Table 2.

By hydro-distillating the leaves and flowering tops, obtaining an essential oil from *Origanum elongatum* is possible with a yield of the order of 2.8% and a yield of the order of 0.7% for *Cupressus atlantica*.

As for organic extracts, the extraction yield for *Origanum elongatum* is about 15.6% and about 9.6% for *Cupressus atlantica* demonstrating that *Origanum elongatum* is richer in extractable compounds by this type of solvent than *Cupressus atlantica*.

Phytochemical screening

The results of the various tests carried out on the two plants to determine the possible presence of the various metabolites are mentioned in Table 3.

The tests performed on the extract of *Origanum elongatum* reveal the presence of gallic tannins, sterols, free quinones and flavonoids. In *Cupressus atlantica*, the tests reveal a different metabolic profile: presence of catechic tannins and sterols (Tab.3).

Moreover, in the two studied plants, the tests performed do not detect any presence of alkaloids and saponins.

Screening for antioxidant activity

The screening of the antioxidant activity was carried out on the various extracts resulting from the two plants *Origanum elongatum* and *Cupressus atlantica*.

Screening on silica plate

The results of the screening of the antioxidant activity performed on the various extracts on silica plate are shown in Fig. 1. 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 deposit δ-Tocopherol.

Analysis of the results shows that the extracts react differently with DPPH. In *Cupressus atlantica*, the essential oil has a low activity because even the reaction time is extended to 240 min with a volume of 8 µl the discoloration of the DPPH remains low. However, the organic extract induces a strong discoloration of the DPPH whatever the volume and time of reaction. This important reduction of the free radical DPPH by the organic extract is visible from the first 15 minutes. In *Origanum elongatum*, the two extracts (essential oil and organic extract) react positively with DPPH, which shows the presence of antioxidant activity. The discoloration of DPPH is very clear and rapid from 15 min of reaction and at low volume.

Spectrophotometric screening

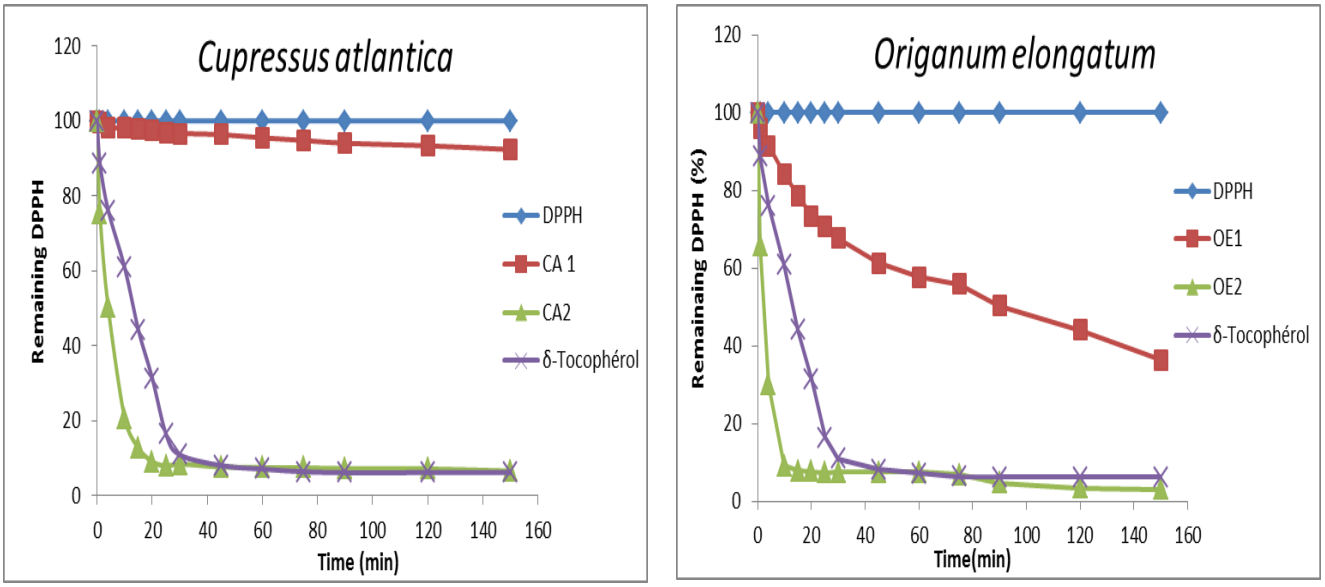

Figure 2: DPPH reduction kinetics (%) in chronological order in the presence of the various extracts of *Cupressus atlantica* and *Origanum elongatum*. *Cupressus atlantica*: CA1: essential oil, CA2: organic extract. *Origanum elongatum*: OE1: essential oil, OE2: organic extract. DPPH 6.10 $^{-5}$ M; δ-Tocopherol at 0.4 mg / ml.

A second screening carried out by spectrophotometry confirms the results obtained on silica plate. The kinetics of degradation of DPPH alone, in the presence of the various extracts at the same concentration (5 mg / ml) or in the presence of δ-Tocopherol, is represented in Fig 2.

In the absence of extracts, the reduction of the DPPH (control without extract) remains stable during the 150 min analysis while the positive control (DPPH plus δ-Tocopherol) shows a significant degradation of the DPPH. The results of the spectrophotometric analyzes in the presence of various extracts seem to confirm those obtained during the first screening on a silica plate.

In *Cupressus atlantica*, the presence of the essential oil in the reaction environment causes the reduction of almost 2.56% DPPH after the first 20 min. At 120 min, this reduction reaches 6.7%. However, in the presence of the organic extract, the DPPH is rapidly and strongly reduced: 90.9% of DPPH after 20 min of reaction. Thus, in the presence of essential oil, the reduction remains low compared to that obtained in the presence of the organic extract.

The studied extracts of *Origanum elongatum* cause a strong degradation of the DPPH present in the environment. The presence of the essential oil induces a reduction of 26.6% of the DPPH after 20 min. This degradation is of the order of 92.3% in the organic extract. Moreover, the presence of the organic extract induces a higher and sharper reduction than that recorded in the presence of δ-tocopherol (68.7%).

DISCUSSION

Antioxydant activity is a highly researched phytotherapeutic property. In this work, the screening of the antioxidant activity is carried out on a silica plate. By using this method, it is possible to evaluate the antioxidant activity of several samples at the same time and in a reduced amount of time and it is a simple and highly effective method widely used[14].

Cupressus atlantica essential oil exhibits a weak antioxidant activity relative to the organic extract which exhibits an interesting antioxidant activity. The work of Salman et al., (2017)[15] and Boukhris et al., (2012)[16] reveal the presence of antioxidant activity in *Cupressus sempervirens* extracts.

However, in *Origanum elongatum*, the studied extracts show a very important antioxidant activity. These findings in *Origanum elongatum* are similar with those found in several other Origanum species such as *Origanum majorana L.*[3], *Origanum compactum*[17], *Origanum glandulosum* Desf.[18], *Origanum vulgare* subsp. Virens[19].

The phytochemical screening of the two studied plants shows that the *Origanum elongatum* contains tannins, flavonoids, sterols and quinones while it does not contain alkaloids and saponins. The findings converge with those of Bendifallah et al. (2015)[20] in Origanum vulgare L with exception of saponins which are not present in our study.

Phytochemical analysis shows that *Cupressus atlantica* is rich in tannins and sterols but does not contain alkaloids and this richness in tannins has also been reported in Cupressus sempervirens L[21].

Secondary metabolites such as polyphenols are known for their antioxidant activities[22].Our results show that *Origanum elongatum* and *Cupressus atlantica* contain flavonoids and tannins. The presence of these metabolites correlates with the presence of antioxidant activity[23-24].

CONCLUSION

At the end of this work, from the two tests using free radical DPPH, we find that the extracts (essential oil and organic extract) of *Origanum elongatum* and *Cupressus atlantica* have an interesting antioxidant activity. The

phytochemical screening of these two plants reveal the richness of these extracts in polyphenols known as antioxidants, which explains in part the presence of antioxidant activity. These two plants are endowed with real antioxidant properties, which makes them used in different fields such as (agro-alimentary, cosmetic, pharmaceutical, and other related fields).

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