

Original Research Paper

In vitro* study on antioxidant ability of two plants *Thymus coloratus* and *Thymus euciliatus

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Article history: Received: May 29th 2022; Revised: December 30th 2022; Accepted: January 08th 2023

Abstract

Thymus ciliatus ssp. *coloratus* and ssp. *euciliatus* are two spontaneous aromatic plants in northern Algeria, and are widely used by local people for their medicinal properties. It is important to underline that spices and herbs have been used not only for flavoring food but also for improving the overall quality of the product and extending the shelf life of foods due to their antioxidant properties. In order to investigate tannins extract with biological activity from local wild plants, two *Thymus* species, *ciliatus* ssp. *coloratus* and ssp. *euciliatus*, were screened for their antioxidant activity. The antioxidant potential of tannins extract was assessed by 1,1-diphenyl-2-picrylhydrazyl free radical scavenging, ferric reducing antioxidant power and the hydrogen peroxide scavenging assays, in comparison with reference antioxidants ascorbic acid and BHA. Phytochemical screening undertaken on studied plants revealed the presence of some chemical compound families, including tannins. Besides, it was observed that the ssp. *euciliatus* tannins have greater activity than the ssp. *coloratus* and higher activity than those of standards. In conclusion, the results suggest that the tannins extract could be used for the future development of antioxidant agents.

Keywords: *Thymus coloratus*, *Thymus euciliatus*, antioxidant activity, tannins, FRAP, DPPH

المخلص

نوعان من النباتات العطرية العفوية من شمال الجزائر ، يستخدمها السكان المحليون على نطاق واسع لخصائصهم الطبية. من المهم الإشارة إلى أن التوابل والأعشاب لم تستخدم فقط لتذوق الأطعمة ، ولكن أيضا لتحسين الجودة الشاملة للمنتج وإطالة العمر الافتراضي للأطعمة بسبب خصائصها المضادة للأكسدة. من أجل دراسة مستخلص العفص النشط بيولوجيا من النباتات البرية المحلية، تم فحص نوعين من الصعتر لأنشطتهما المضادة للأكسدة. تم تقييم إمكانات مضادات الأكسدة لمستخلص العفص من خلال مسح الجذور الحرة بواسطة DPPH و FRAP، مقارنة بمضادات الأكسدة المرجعية. كشفت الفحوصات الكيميائية النباتية التي أجريت على النباتات المدروسة عن وجود عائلات من المركبات الفينولية ، بما في ذلك العفص. علاوة على ذلك، لوحظ أن العفص في *ssp euciliatus* لها نشاط أعلى من نشاط *ssp coloratus* ونشاط أعلى من تلك المعايير المرجعية. في الختام، تشير النتائج إلى أنه يمكن استخدام مستخلص العفص في التطوير المستقبلي للعوامل المضادة للأكسدة

الكلمات المفتاحية : *Thymus euciliatus* ، *Thymus coloratus* ، نشاط مضادات الأكسدة ، العفص ، FRAP ، DPPH

Introduction

The plant kingdom has proven to be the most useful in the treatment of diseases, and they provide an important source of all the world's pharmaceuticals.

Nowadays, it is commonly accepted that disturbances in regulating free radical processes in the organism are among the factors leading to serious pathologies such as radiation damage, atherosclerosis, myocardial infarction, diabetes, cancer, spoilage of foodstuff and others (Pan et al., 2008). Not neutralized, the excess free radicals lead to oxidative stress, violate metabolism, and cause the aforementioned disorders. For this reason, chemotherapy association with antioxidants finds increased use in the treatment of such diseases.

During the past decade, commercial antioxidants used were synthetic antioxidants such as 2,3-tert-butyl-4-methoxyphenol (BHA), 2,6-di-tert-butyl-4-methylphenol (BHA), the tert-butyl hydroquinone

(TBHQ) and propyl gallate (PG) suspected that containing some toxicity and responsible of carcinogenesis and damage in the liver. Hence the importance of antioxidants has focused on the research development and isolation of natural antioxidants from plants to replace those obtained by synthesis (Pan et al., 2008; Tawaha et al., 2007; Atmani et al., 2009). A majority of isolated antioxidants from plants are composed of polyphenols. In this family, we have a class of secondary metabolites found responsible for numerous biological activities.

Thymus ciliatus ssp. *coloratus*, an aromatic plant, is spontaneous and widespread in the Mediterranean and northern Algeria. These two Lamiaceae family sub-species grow as very compact sub-shrubs, with many branches, twisted and standing (Delanch, 1978). They are widely used by local people for their medicinal properties such as antifungal and antibacterial.

Hence, this study aims to determine the phytochemical constituent and investigate the antioxidant properties of tannins extract isolated from *Thymus coloratus* and *T. euciliatus* to ascertain the rationale for its use in traditional medicine.

Material and methods

Plant Material

Thymus coloratus was picked at mature age during May 2021 in the Beni Mester region and *Thymus euciliatus* in July in the Aïn Fezza region. The two study areas are located in Tlemcen, a city in northwestern Algeria.

The plant material was then collected and dried away from moisture and sunlight for fifteen (15) days on the ground in jute bags.

Phytochemical tests

The plant's chemical composition was carried out using solubility tests, staining reactions, and precipitation as well as by examination under ultraviolet light. The presence of tannins is highlighted by adding 1 mL of ethanol extract, 2 ml of water, and 2 to 3 drops of ferric chloride (FeCl_3) diluted to 1%. The appearance of a blue-black color indicates the presence of gallic tannins and green to blue-green of the catechic tannins (Trease et al., 1987).

Extraction of tannins

The tannin extraction was obtained using the Zhang et al. method, which consists of maceration of 2.5 g of the crushed two plants per 50 mL of acetone/distilled water mixture for three (3) days (Zhang et al., 2008).

Antioxidant Activity

Reduction of Iron: FRAP (ferric reducing antioxidant power)

The iron-reducing activity of our extracts was determined using the method described by Pan et al. (2008). Based on the chemical reaction of reduction of ferric ions (Fe^{3+}) present in the complex $\text{K}_3\text{Fe}(\text{CN})_6$ as ferrous ions (Fe^{2+}) (Pan et al., 2008).

Scavenging of free radical DPPH

DPPH (2,2-diphenyl-1-picrylhydrazyl) is generally used as the substrate for fast and direct evaluation of the antioxidant activity due to its free radical form stability and simplicity of the analysis. It absorbs in the visible wavelength of 515 nm (Bozin et al., 2008). The experimental protocol followed to study the activity of scavenging DPPH free radicals is that described by Chaouche et al. (2020).

Scavenging of hydrogen peroxide (H_2O_2)

One of the most common methods for evaluating the scavenging ability of hydrogen peroxide is based on the absorption of this molecule in the UV range. The scavenging compounds are responsible for the

decrease in the concentration of H_2O_2 and thus the value of the absorbance at 230 nm of the latter decreases also. The method used in this test is that described by Ruch et al. (1989).

Results

Phytochemical tests

The phytochemical screening revealed a very strong presence of tannins in the two plants confirmed by a positive reaction using a ferric chloride solution which gave a blue-greenish color characterizing catechin tannins.

Study of antioxidant activity

The *in vitro* antioxidant activity of our extracts was evaluated by three different methods namely FRAP (for ferric reducing antioxidant power), DPPH, and hydrogen peroxide scavenging activity assays.

Reduction of Iron

In our work, we tested the tannins extract from the two plants' aerial parts. The results in Figure 1 shows that the reduction capacity is proportional to the increasing concentration of extracts.

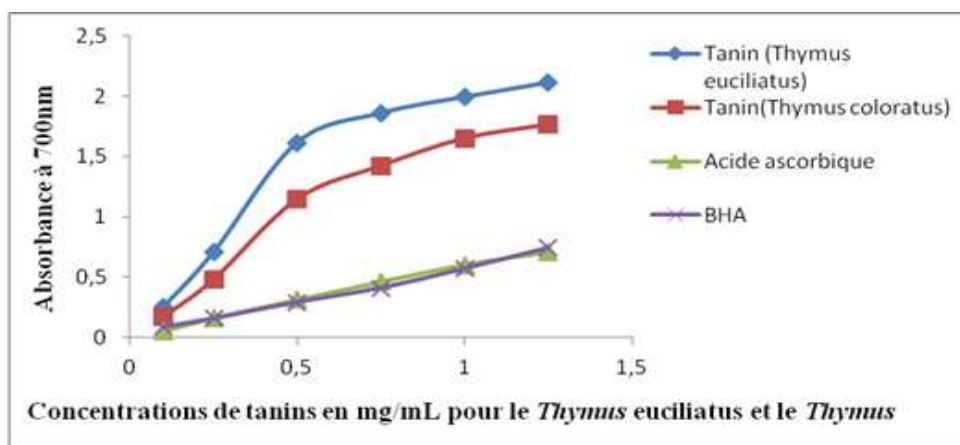


Figure 1: Tannins reducing powers for BHA and ascorbic acid and *Thymus euciliatus* and *Thymus coloratus*.

At a concentration of 1.25 mg/mL *T. euciliatus*, tannins extract is more powerful (OD = 2.110) than *T. coloratus* tannins extract (OD = 1.764) (Figure 1). We noted that the two plants have excellent antioxidant activities than those of ascorbic acid and BHA.

Scavenging of free radical DPPH

The antioxidant activity of our extract vis-à-vis the radical DPPH is measured spectrophotometrically by following the reduction of the radical which is followed by the transition from purple to yellow measurable at 515 nm.

Inhibition ratio of free radical DPPH for reference substances

Figures 2 and 3 show the calibration curves of BHA and ascorbic acid. for BHA

For comparative purposes, ascorbic acid and BHA, standard antioxidants, showed significant anti-radical activities with IC_{50} respectively 1.12 mg/mL and 1.61 mg/mL.

Percent inhibition of free radical DPPH for *T. coloratus* and *T. euciliatus* tannins.

In this case, the study focuses on the antioxidant activity of extracts from the two plants' tannins in order to know the most active. The following figures (Figures 4 and 5) report the percentage of

inhibition obtained with the concentrations used in the studied plants. Generally, tannins of both studied plants cause a significant decrease in absorbance at 515 nm depending on their concentrations. *Thymus euciliatus* tannins show a radical DPPH scavenging effect higher than *Thymus coloratus* tannins (74% against 63%). Also, their activities significantly exceed those of standard antioxidants.

Scavenging of hydrogen peroxide (H_2O_2)

In this study, the toxic molecule is subjected to an antioxidant effect (concentration 0.1 g/mL of tannin extract) and follows the course of the reaction versus time (40 minutes). The percentage of H_2O_2 scavenging is given by:

$$\% H_2O_2 = [(AC - AT) / AC] \times 100$$

Where AC is : Absorbance of control and AT is : Absorbance of the test.

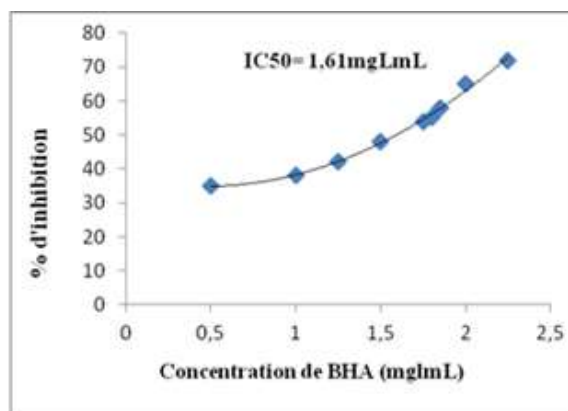


Figure 2: Percentage of inhibition of DPPH free radical according to the different concentrations used for BHA

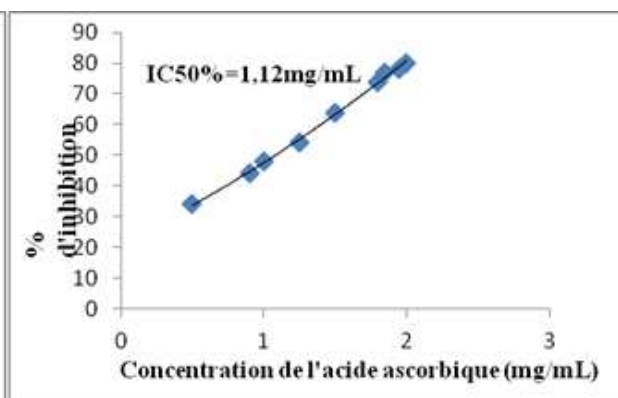


Figure 3: Percentage of inhibition of DPPH free radical according to the different concentrations used for ascorbic acid

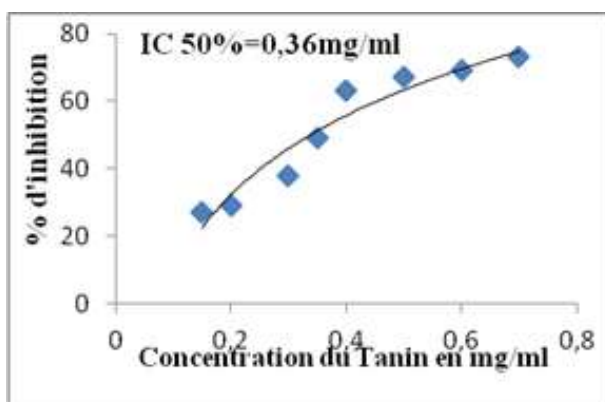


Figure 4: Percentage of inhibition of free radical DPPH depending on the different concentration used for *Thymus coloratus* tannins.

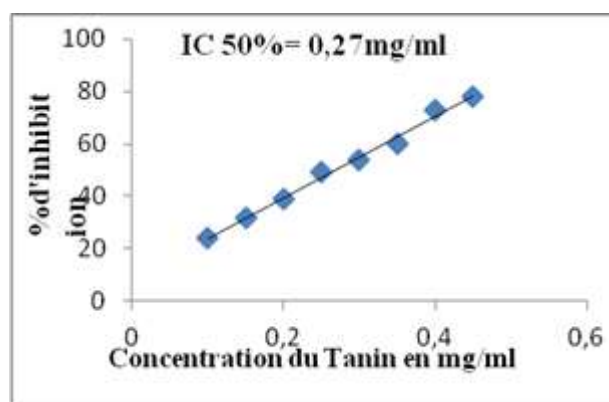


Figure 5: Percentage of inhibition of free radical DPPH depending on the different concentration used for *Thymus euciliatus* tannins

The results in Table 01 show that during the kinetic (40 minutes) absorbance at 230 nm decreases with time. This shows that the latter, due to hydrogen peroxide, is converted to H_2O by antioxidants (tannins) present in both plants studied.

Table 01: Kinetics of hydrogen peroxide scavenging of tannins extracts from both plants.

	Temps (min)				
	0	10	20	30	40
BHA (%)	74,08 ± 1,4	67 ± 1,1	64 ± 1,2	48 ± 0,9	33 ± 0,8
<i>Thymus euciliatus</i> (%)	77 ± 2.1	67,62 ± 1,8	65,01 ± 1,7	60,25 ± 1,1	56 ± 0,9
<i>Thymus coloratus</i> (%)	58,4 ± 1,1	53,28 ± 1,1	40 ± 0,7	34 ± 0,6	28,08 ± 0,6

Figure 6 shows that the concentration of 0.1 µg/mL and at the initial time (t_0), *Thymus euciliatus* tannins have an activity (77%) similar to that of BHA (74.08 %) and significantly higher than that of *Thymus coloratus* (58.40 %).

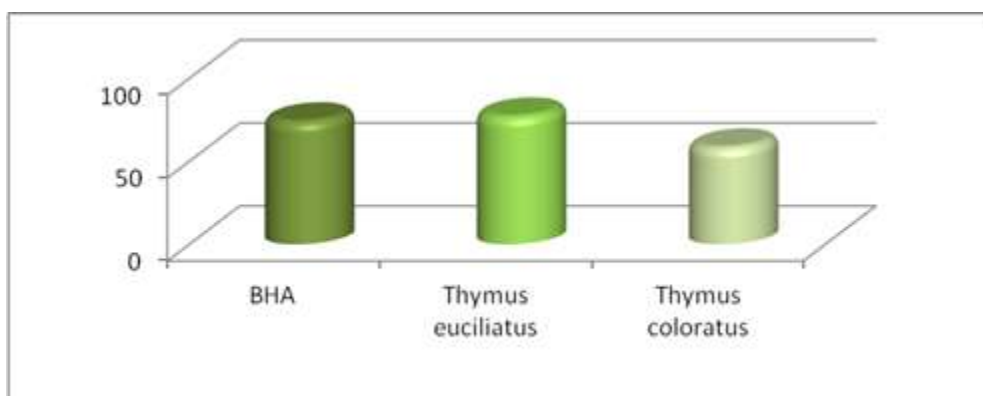


Figure 6: Activities of scavenging hydrogen peroxide to tannins extract from both plants at the initial time (t_0)

Discussion

The ferric reducing antioxidant power is a fast analysis of the antioxidant activity, reproducible and easy to perform. This method is based on the phenolic compound's ability to reduce ferric iron Fe^{3+} to ferrous iron Fe^{2+} . The reduction power is one of the antioxidant mechanisms (Karağözü et al., 2008).

In our work, we tested the tannins extract from the two plants' aerial parts. The results in Figure 1 shows that the reduction capacity is proportional to the concentration increase of our samples (Oztürk et al., 2007; Su et al., 2008; Liu et al., 2009).

So, the most interesting antioxidant activity is that of *Thymus euciliatus* and this can probably be explained by the presence of compounds capable of reducing iron.

It should be noted that the reduction capacity is proportional to the increase in the concentration of our samples. This is confirmed by many authors (Su et al., 2008; Benhammou, 2006).

The reducing ability of a compound may be used as a significant indicator of its potential antioxidant activity (Yang et al., 2008). Many studies show that there is a direct correlation between antioxidant activity and the reducing power of some plant components (Yildirim et al., 2008).

The DPPH radical is one of the most commonly used substrates for fast and direct evaluation of antioxidant activity due to its stability in the radical form and simplicity of the analysis (Bozin et al., 2008). The effect of antioxidants on DPPH is thought to be due to their hydrogen-donating ability (Baumann et al., 1979). Radical scavenging activities are very important to prevent the deleterious role of free radicals in different diseases, including cancer. DPPH free radical scavenging is an accepted mechanism by which antioxidants act to inhibit lipid peroxidation. The results of the DPPH method are consistent with those in the literature (Tian et al., 2009). It became clear that *Thymus euciliatus* tannins present the highest antioxidant activity compared to *Thymus coloratus* tannins (74%

against 63%). Besides, their activities significantly exceed those of standard antioxidants vitamin C and BHA for DPPH scavenging activity.

The hydrogen peroxide can cross cell membranes and thus oxidize and damage many cellular components. The decrease in this percentage is proportional to the absorbance. The latter is due to the H_2O_2 molecule that absorbs the UV at 230 nm (Malgalhaes et al., 2008). The hydrogen peroxide itself is not very reactive but is sometimes toxic to the cell because it is the source of hydroxyl radicals in the cells.

Therefore, the removal of H_2O_2 is very important for antioxidant defense in the cell. Indeed, there is evidence that dietary polyphenols protect animal and bacterial cells from H_2O_2 induced toxicity. Our results indicate that the antioxidant compounds (tannins) in our extracts are good electron donors, thereby accelerating the conversion of H_2O_2 to H_2O and thus eliminating H_2O_2 (Atmani et al., 2009; Shon et al., 2007).

Conclusion

The last decades are marked by a particular interest in the development of medicinal plants as a source of medicinal interest in natural bioactive substances. Thus, many studies have focused more on the therapeutic effects of naturally occurring antioxidants.

This study focuses on the *Thymus euciliatus* and *Thymus coloratus* species belonging to the Lamiaceae family, one of the most important families in the Algerian flora and among the most used by traditional healers. It helped to highlight through a phytochemical screening the presence of tannins in both plants.

The *Thymus coloratus* and *Thymus euciliatus* tannins' antioxidant power is determined by three different techniques. The ability to scavenge DPPH radical and reduce iron. This approach shows that the *Thymus euciliatus* has a higher activity than the *Thymus coloratus*.

Indeed, *Thymus euciliatus* tannins have a fairly high antioxidant activity with an IC_{50} value of about 0.27 mg/mL followed by those of *Thymus coloratus* with 0.36 mg/mL. The FRAP analysis reveals an important activity in the *euciliatus* species followed by *coloratus*, ascorbic acid and BHA. The scavenging of hydrogen peroxide shows a more pronounced activity among *Thymus euciliatus* than *Thymus coloratus* and BHA (control).

We note that there is a certain similarity between the results obtained by FRAP analysis and those determined by the DPPH method.

Funding:

The authors declare that no funding was procured for carrying out the research described in this article.

Conflicts of interest/Competing interests: Not applicable.

Availability of data and material: Not applicable.

Author contributions

LAZOUNI Hamadi Abderrahmane and KHOLKHAL Fatéma : Designed the study, carried laboratory work, literature search, and drafting the article

CHAOUCHE Tarik Mohammed : Revised, participated in the statistical analysis and improved the paper

CHABANE SARI Meriem: Contributed to the interpretation of results

CHABANE SARI Daoudi: Final approval of the version to be submitted

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