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Antioxidant and antimicrobial activities of flavonoids extracted from *Thymus ciliatus* (Desf.) Benth.

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ABSTRACT

The antioxidant and the antimicrobial activities of flavonoids extracted from Thymus ciliatus (Desf.) Benth., an endemic species in Algeria were investigated. The radical scavenging capacity using 2,2- diphenyl-1-picryhydrazyl (DPPH) and the reducing power test were determined to evaluate the antioxidant activity of the samples. The antimicrobial activity was tested with three bacterial strains and three fungi including yeast (Escherichia coli ATCC 25922, Salmonella typhimurium ATCC 13311, Staphylococcus aureus ATCC25923, Aspergillus niger 2CA936, Aspergillus flavius NRRL3357 and Candida albicans ATCC1024). Our results showed a very strong antioxidant activity and an interesting antifungal power.

Key words: antioxidant, antimicrobial, *Thymus ciliatus*, flavonoids,

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INTRODUCTION

Thymus genus (Lamiaceae) is widely distributed; it comprises eight sections distributed into about 220 species [1]. In Algerian flora, there are 12 Thymus species from which 9 are endemic [2], among which *Thymus ciliatus* (Desf.) Benth., which is an endemic species from North Africa. This species includes three subspecies: ssp. eu-ciliatus Maire, ssp. coloratus (Boiss. et Reut.) Batt. and ssp. munbyanus (Boiss. et Reut.) Batt. [2].

In North African folk medicine, *Thymus* plants are used as remedies in various diseases e.g. bronchitis, pulmonary infection, flu, cough and some gastrointestinal disorders [1].

Flavonoids are hydroxylated phenolic substances and are known to be synthesized by plants in response to microbial infection [3]. Their activities are structure dependent. The chemical nature of flavonoids depends on their structural class, degree of hydroxylation, other substitutions and conjugations, and degree of polymerization [4]. Recent interest in these substances has been stimulated by the potential health benefits arising from the antioxidant activities of these polyphenolic compounds. Functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions [5] [6].

Since these secondary metabolites are synthesized by plants in response to microbial infection, it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms [7] [8].

The purpose of this study is to investigate the therapeutic sides of the flavonoids of this endemic species to find a new molecules able to reduce the use of synthetics medicines.

MATERIALS AND METHODS

Flavonoids extraction

The dried aerial plant matrix was milled into coarse powder, then 10 g were defatted three times with petroleum ether (each 40 ml) for 3 hours, at 50 C° [9]. The powder was taking up again three times with 70% ethanol (raw material: solvent ratio was 1: 10) for 90 minutes at 100°C. The extracts were pooled and concentrated in vacuum to collect the aqueous residue (10 ml), which was extracted with chloroform, and then acidified with 20% H_2SO_4 (pH = 5) and extracted with ethyl acetate. The appearance of an interphase precipitate was observed upon extraction with ethyl acetate. The ethyl acetate fraction was taken as a flavonoids fraction for our experiment [10].

Determination of total flavonoids contents

The flavonoids contents in the extracts were estimated by the Aluminium chloride solution according to the method described by Bahorunet *al.* (1996) [11]. Briefly, 1 ml of the methanol solution of the extract was added to 1 ml of 2% $AlCl_3$ in methanol. After 10 minutes, the absorbance was determined at 430 nm. Quercetin was used as a standard. Results were expressed as mg equivalent Quercetin per gram of extract (mg EQ/GE)

DPPH Assay

The donation capacity of extract was measured by bleaching of the purple-colored solution of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato *et al*. (1998) [12].

One milliliter of the extract at different concentrations was added to 0.5 ml of a DPPH-methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 minutes in the dark. The absorbance of the resulting solution was then measured at 517 nm. The antiradical activity was expressed as IC_{50} (micrograms per milliliter), the antiradical dose required to cause a 50 % inhibition. A lower IC_{50} value corresponds to a higher antioxidant activity. The ability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH\ scavenging\ effect\ (\%) = [(A_0 - A_1)/ A_0] \times 100$$

Where A_0 is the absorbance of the control at 30 minutes and A_1 is the absorbance of the sample at 30 minutes. BHT was used as a standard and samples were analyzed in triplicate [13].

Reducing power

The reducing power was determined according to the method of Oyaizu (1986), [14]. The extract (0.5–10 mg/ml) in methanol (2.5 ml) was mixed with 2.5 ml of 200 mmol/l sodium phosphate buffer (pH 6.6) and 2.5 ml of 10 mg/ml potassium ferricyanide. The mixture was incubated at 50 C° for 20 minutes; after, 2.5 ml of 100 mg/ml trichloroacetic acid were added, the mixture was centrifuged at 200g for 10 minutes.

The upper layer (5 ml) was mixed with 5 ml of deionized water and 1ml of 1 mg/ml ferric chloride, and the absorbance was measured at 700 nm against a blank.

A higher absorbance indicates a higher reducing power. EC_{50} value (mg extract/ml) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis. Ascorbic acid was used as standard [15].

Antimicrobial activity:

The antimicrobial activity was carried out with the extract following the test below:

Test strains and culture media

Strains of bacteria were obtained from the American Type Culture Collection, three bacterial strains were tested: *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 13311 and *Staphylococcus aureus* ATCC25923.

Two funguses: *Aspergillus niger* 2CA936 and *Aspergillus flavus* NRRL 3357; and one yeast: *Candida albicans* ATCC1024.

Muller Hilton agar was used for bacteria culture, the potato dextrose agar for funguses culture and Sabouraud for yeast.

Anti-bacterial Activity

Agar disc diffusion method [16] [17] was employed for the determination of antibacterial activities of flavonoids extracted from *Thymus ciliatus* (Desf.) Benth.

Briefly, a suspension of the tested microorganism (0.1 ml 10^8 cells per ml) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 10 µl of 100 µg/ml of the extract and placed on the inoculated plates.

These plates were incubated at 37 C° for 24 h. Gentamicin (10 µg/disc) was used as a standards and dimethylsulfoxide DMSO as a control.

The antibacterial activity was determined by measuring of inhibition zone diameters (mm) and was evaluated according the parameters suggested by Alves *et al*. (2000) [18]:

- ✓ <9 mm, inactive ;
- ✓ 9–12 mm, less active ;
- ✓ 13–18 mm, active;
- ✓ >18 mm, very active.

Antifungal activity

The antifungal activity was tested by disc diffusion method with modifications [16]. The potato dextrose agar plates were inoculated with each fungal culture (*Aspergillus niger* 2CA936, *Aspergillus flavus NRRL 3357*), 8 days old by point inoculation.

The spore suspension was prepared in an emulsion of 0,5 % tween 80, adjusted to a concentration of 2-3 × 10^6 spores/ml, corresponding to 0.15 to 0.17 absorption when a spectrophotometer set at 530 nm was used [19]. However, *Candida albicans* ATCC1024 suspension is obtained from a culture in Sabouraud 24 h 37 C° adjusted to 10^5 cfu / ml.

One hundred microliter of suspension was placed over agar in Petri dishes and dispersed. Then, sterile paper discs (6 mm diameter) were placed on agar to load 10 µl of each sample at 100 µg/ml.

Nystatin 100µg, clotrimazon 50 µg and amphotericin 100 µg were used as a standards and dimethylsulfoxide DMSO as a control. Inhibition zones were determined after incubation at 27 C° for 48 h.

Statistical analysis

Results were expressed as the mean ± standard deviation. Data was statistically analyzed using t test of Student with the criterion of P values < 0.05 to determine whether there were any significant differences between flavonoids extract of *Thymus ciliatus (*Desf.) Benth.*,* and standards, using Graphpad prism 5 Demo Software.

RESULTS AND DISCUSSION

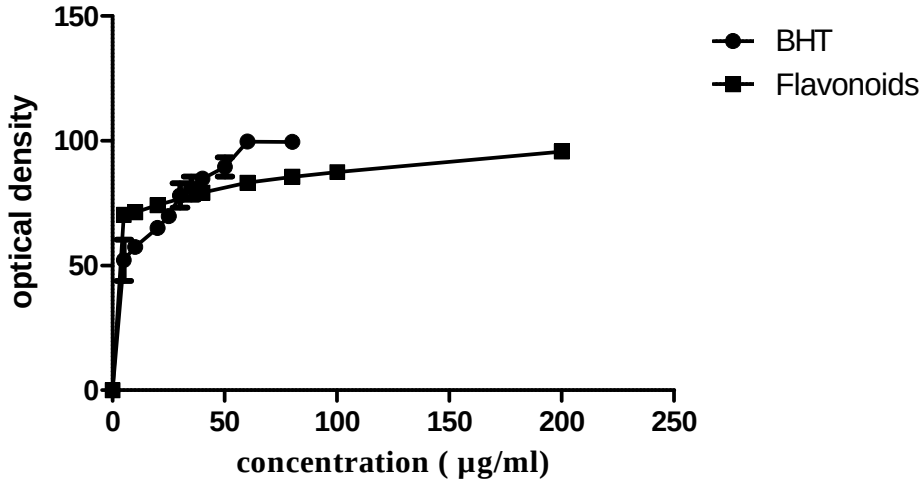

Figure 1: DPPH radical scavenging activity of flavonoids extracted from Thymus ciliatus (Desf.) Benth.

The yield of flavonoids extract reached 3.8 % and the quantification of pure flavonoids gave 134.34±8.97 mg EQ/GE.

The DPPH radical scavenging properties of the extract (figure 1) seem to indicate that flavonoids have a very strong activity with IC_{50} of 5.39±0.10 µg/ml against 8.76±0.69 µg/ml for BHT. Statistic comparison indicate that there is no significant difference between standard and the extract.

The role of antioxidants in the inhibition of antioxidant processes occurring in living organisms consists of: scavenging free radicals and quenching singlet oxygen, disconnection of radical reactions, chelate metals which catalyze the oxidation process, inhibition of certain enzymes (eg., oxidases). Flavonoids are active in all these processes [20]

It is known that only flavonoids with a certain structure and particularly hydroxyl position in the molecule can act as proton donating and show radical scavenging activity [21] [22].

The effectiveness of flavonoids in DPPH radical scavenging depend largely on their structure, hydrophobicity, biological and oxidative activity. The ability and disconnection of radical chain reactions by flavonoids is mainly dependent on the presence in B ring of at least two *o*-hydroxyl groups. It enables the formation of intramolecular hydrogen bond between hydroxyl groups, which increases the stability of the phenoxyl radicals [23].

Figure 2 describes the reducing power of flavonoids extracted from *Thymus ciliatus* (Desf.) Benth. As it can be seen, flavonoids showed an important antioxidant property with EC_{50} of *33.71±0.22 µg/ml*, as ascorbic acid which make an EC_{50} equal to *8.46±0.09 µg/ml* and statistic treatment showed a significant difference.

High reducing power of flavonoids suggested their remarkable potency to donate electrons to reactive free radicals, thus converting them into more stable non-reactive species and finally terminate the free radical chain reaction [24]. It was confirmed that the hydroxyl groups at C-3' and C-4' of the B-ring to be more active in reducing iron concentration [25].

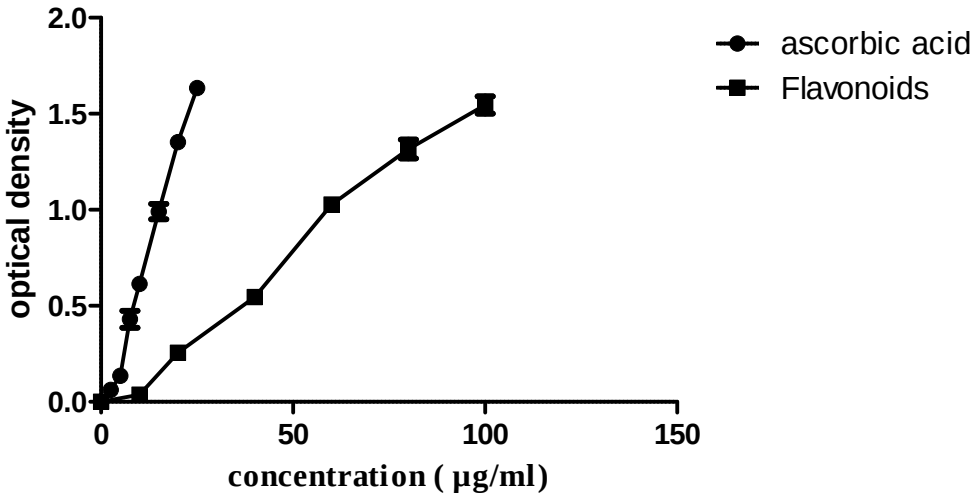

Figure 2: reducing power activity of flavonoids extracted from Thymus ciliatus (Desf.) Benth.

The results obtained from antimicrobial assay are presented in Table 1 and 2 at a concentration of 1 mg/disc.

Table 1: Inhibition zones in millimeter for standards, extract and control against bacterial strains

	Escherichia coli ATCC 25922	*Salmonella typhimurium ATCC 13311*	*Staphylococcus aureus ATCC25923*
standard	*18,50±0,41*	*19,17±0,24*	*27,67±0,47*
Flavonoids	*No inhibition*	*9,33±0,47*	*14,84±1,99*
Control	*No inhibition*	*No inhibition*	*No inhibition*

According to Table 1, the flavonoids of *Thymus ciliatus* (Desf.) Benth., showed an important antibacterial activity against *Staphylococcus aureus* but a low activity against *Salmonella typhimurium*.

Statistic treatment indicate a very highly significant difference between flavonoids and standard.

Table 2: Inhibition zones in millimeter for standards, extract and control against fungi strains

	Aspergillus niger 2CA936	*Aspergillus flavius NRRL3357*	*Candida albicans ATCC1024.*
Nystatin	*9,40±0,22*	*15,53±0,79*	*9,29±0,19*
Clotrimazon	*15,85±0,32*	*23,86±1,15*	*44,28±0,49*
Amphotericin	*17,55±0,14*	*16,20±1,19*	*15,58±0,12*
Flavonoids	*100 % of inhibition of mycelia growth*	*No inhibition*	*No inhibition*
control	*No inhibition*	*No inhibition*	*No inhibition*

Table 2 indicate a very interesting capacity of the flavonoids extracted from *Thymus ciliatus* (Desf.) Benth., to inhibit the growth of mycelia of *Aspergillus niger*. Statistic treatment indicate a very significant difference between flavonoids and standard.

The flavonoids are increasingly becoming the subject of anti-infective research. Several flavonoids have been known to possess antibacterial and antifungal activities [26].

It has been reported that activity against gram positive bacteria such as *Staphylococcus aureus* is shown by flavonoids with a hydroxyl group in the ring B [27].

The inhibition of microorganisms by phenolic compounds may be due to iron deprivation or hydrogen bonding with vital proteins such as microbial enzymes [28].

CONCLUSION

The genus *Thymus* belonging to the family Lamiaceae (Labiatae) was widely investigated especially the endemic ones of Algeria, and for the best of our knowledge, this is the first time which the pure flavonoids extracted from *Thymus ciliatus (Desf.) Benth,* were investigated for their antioxidant and antimicrobial powers.

The results were very interesting in both activities with an important yield of flavonoids. This needs more exhaustive chemical research to develop a new medicines or fungicide with preserving the harmonium interaction of the natural molecules composition and to produce industrially a new natural products.

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REFERENCES

[1] J Bellakhdar, *Ibiss press*, France, **1997**,
[2] P Quezel , S Santa., C.N.R.S., Paris, France, **1963** , p1170
[3] R A Dixon, P M Dey, C J Lamb, *Advances in Enzymology and Related Areas of Molecular Biology*, **1983** 55, 1–136.
[4] E H Kelly, R T Anthony, J B Dennis, *Journal of Nutritional Biochemistry*, **2002**, 13 (10), 572–584.
[5] S Kumar, A Mishra, A K Pandey, *BMC Complementary and Alternative Medicine*, **2013**, 13, 120.
[6] S Kumar, A K Pandey, *Vegetos*, **2013**, 26, 301–307.
[7] JB Harborne, *Chapman &Hall*: London, **1993**.
[8] MM Cowan, *Clin. Microbiol. Rev*, **1999**, 12, 564-582.
[9] B He-Long, W Jing, L Chun-Ming, L Li, *Journal of the Chinese Chemical Society*, **2010**, 57, 1071-1076.
[10] N K Chirikova, D N Olennikov, L M Tankhaeva, *Russian Journal of Bioorganic Chemistry*, **2010**, 36 (7), 915–922.
[11] T Bahorun, B Gressier, F Trotin, C Brunete, T Dine , J Vasseur, J C Gazin, M Pinkas, M Luycky, M Gazin, *Arzneim Forsch / Drug Res*, **1996**, 1-6.
[12] T Hanato, H Kagawa, T Yasuhara, T Okuda, *Chemical & Pharmaceutical Bulletin*, **1998**, 2090–2097.
[13] R I Bettaieb, S Bourgou, I Ben Slimen Debez, I Jabri Karoui, I Hamrouni Sellami, K Msaada, F Limam, B Marzouk, *Food Bioprocess Techno* , **2011**, 1007.
[14] M Oyaizu, *Japanese Journal of Nutrition*, **1986**, 307–315.
[15] S J Huang, J L Mau, *Swiss Society of Food Science and Technology*, **2006**, 39 , 707–716.

[16] NCCLS (National Committee for Clinical Laboratory Standards), *9th International Supplement*, **1999**, M100-S9.
[17] NCCLS (National Committee for Clinical Laboratory Standards), *6th ed. Approved Standard*, **1997**, M2-A6.
[18] T M A Alves, AF Silva, M Brandão, TSM Grandi, E F A Smânia, Jr A Smânia, C L Zani, *Memórias do Instituto Oswaldo Cruz*, **2000**, 95: 367–373.
[19] D Yazdani, M A Zainal Abidin, Y H Tan, S Kamaruzaman , I B Jaganath, *Journal of Medicinal Plants Research*, **2012**, 6(42): 5464-5468.
[20] R J Nijveldt, E Van Nood, D N Van Hoorn, P G Boelens, K Van Norren, P A M Van Leeuwen *Am. J. Clin. Nutr*, **2001**, 74: 418.
[21] B Nickavar, M Kamalinejad, H Izadpanah, *Pak J Pharm Sci*. **2007**, 20(4), 291-4.
[22] A Wojdyłoa, J Oszmiańskia, R Czemerysb, *Food Chemistry*, **2007**, 105 (3), 940–949.
[23] M Majewska, M Skrzycki, M Podsiad, H Czeczot, *Acta Poloniae Pharmaceutica - Drug Research*, **2011**, 68 (4), 611-615,
[24] X -Q Zha, J -H Wang, X -F Yang, H Liang, L –L Zhao , S -H Bao, J –P Luo, Y -Y Xu, B -B Zhou, *Carbohyd. Polym*, **2009**, 78, 570–575.
[25] J F Moran, R V Klucas , R J Grayer, J Abian., M Becana, *Free Radic. Biol. Med*, **1997**, 22: 861–870.
[26] N Jayshree, N Narayanan, L Sriram, *Asian J Pharm Cl in Res*, **2012**, 5 (1), 101-103
[27] A Martin, M Camacho, F Portaels, JC Palomino, *Antimicrob Agents Chemother*, **2003**, 47(11), 3616 – 3619.
[28] A Scalbert, *Phytochemistry*, **1991**, 30, 3875-3883.