Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (1):118-121
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Antioxidant and antimicrobial activities of an endemic desert species *Thymelea microphylla* Coss. et Dur.

Labib Noman[1], Amar Zellagui[1], Youcef Hallis[1], Ayse Sahin Yaglioglu[2], Ibrahim Demirtas[2], Noureddine Gherraf[1] and Salah Rhouati[1]

[1]*Laboratory of Natural Products and Organic Synthesis, Department of Chemistry, Facultof Science, University of Mentouri–Constantine, Algeria*
[2]*Laboratory of Plant Research, Department of Chemistry, Faculty of Science, Gaziosmanpaşa University, Uluyazi Campus, Cankiri Karatekin University, Cankiri, Turkey*
[2]*Centre for Arid Areas (CRSTRA), Biophysical Station, Touggourt, Algeria*

ABSTRACT

Aerial parts of Thymelaea microphylla extracted with CH_2Cl_2–MeOH (1:1) at room temperature. The crude extract was subjected to evaluate the antimicrobial activity by disc diffusion method and tested against Gram-positive and Gram-negative bacteria and showed a strong antibacterial activity against Staphylococcus blanc at 8mg/mL. In addition this crude extract was screened for their possible in vitro antioxidant activity by DPPH free radical-scavenging test. The findings showed that the percentage of reduction is 72 % at 1/10 M. which were exerted highest antioxidant activity than standard vitamin C.

Keywords: *Thymelea microphylla*, Antioxydant activity, Antimicrobial activity

---

INTRODUCTION

Antioxidants can protect the human body from free radicals and ROS effects. They retard the progress of many chronic diseases as well as lipid peroxidation [1]. Also, antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods [2]. On the other hand Increasing resistance of microorganisms against available antimicrobial agents is of major concern among scientists and clinicians worldwide. In general, it is observed that pathogenic viruses bacteria, fungi, and protozoa are more and more difficult to treat with the existing drugs [3]. A great number of plants worldwide showed a strong antioxidant activity [4]. This antioxidant capacity can be explored by using plants as a source of antioxidants to prevent the rancidity and oxidation of lipids. [5].

The genus *Thymelaea* Mill includes 31 species. In Algeria, it is represented by 7 species among which *Thymelaea microphylla* Coss and Dur. (endemic plant). *Thymelaea mirophylla* Coss. et Dur. Growing in arid and desert [6]. The Genus *Thymelaea* is known for its variety of secondary metabolites such as tigliane, daphnane, coumarins, flavonoids, lignans, sterols, triterpenes, saponins, tannins, and essential oil. [7, 8, 9] as well as their biological activities [10].

On our continuation of investigation carried out on the *Thymelaea microphylla* Coss. and Dur. (endemic plant) growing in El oued (East Algerian sahara). (Labib Noman *et al*., 2010), the main objectives of this study were:
(i) To evaluate the antimicrobial activity of the crude extract by the disk diffusion method.
(ii) assessment of the *in vitro* scavenging activity by DPPH test.

MATERİALS AND METHODS

2.1. Plant material

The aerial parts of *Thymelaea microphylla* Coss. and Dur. were collected in the end of March 2010 (flowering stage) in **El-Oued**, south-east of Algeria. The plant was identified by Dr. Chahma A. M., University of Ouargla. Fresh aerial parts were dried to constant weight at room temperature. A voucher specimen was deposited at the chemistry Department, University of Mentouri-Constantine 1 under the code number ZA 107.

2.2. Microorganisms

All of the bacteria (standart strains: *E. Coli atcc 25922, Staphylococcus blanc ATCC 27853, Staphylococcus aureus* ATCC25923) were obtained from Bacteriology Laboratory Constantine Hospital University (C.H.U), while the fungus strain *Aspergillus niger* was isolated in microbiology laboratory, department of biology, Constantine 1 University.

2.3. Extraction and isolation

Aerial parts of *Thymelaea microphylla* Coss. and Dur. **(**2200 g) was crushed and extracted with CH_2Cl_2–MeOH (1:1) at room temperature. The extract was concentrated in vacuo to obtain a residue (103.7 g) [11] .

2.4. Antimicrobial activity

The anti-microbial activity test was carried out on crude extract of a *Thymelaea microphylla* Coss. and Dur. using disk diffusion method [12] against three human pathogenic bacteria, including Gram positive, Gram-negative bacteria and one fungus *Aspergillus niger.* The bacterial strains were first grown on Muller Hinton medium (MHI) at 37 ˚C for 24 h prior to seeding on to the nutrient agar but the *Aspergillus niger* at 30 ˚C for 48 h. A sterile 6-mm-diameter filter disk (Whatman paper n° 3) was placed on the infusion agar seeded with bacteria, and each extract suspended in water was dropped on to each paper disk (40 µl per disk) for all of prepared concentrations (8000µg/ml, 4000µg /ml, 2000µg/ml, 1000µg/ml, 500µg/ml, 250µg/ml). The treated Petri disks were kept at 4 ˚C for 1 h, and incubated at 37 ˚C for 24 h. The antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the disks. Each experiment was carried out in triplicate.

.

2.5. Antioxidant activity

The free radical-scavenging activity of *Thymelaea microphylla* Coss. and Dur. crude extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. In this assay, the purple chromogen radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•) is reduced by antioxidant/reducing compounds to the corresponding pale yellow hydrazine [13]. The scavenging capacity is generally evaluated in organic media by monitoring the absorbance decreases at 515 to 528 nm until the absorbance remains constant. 0.1 mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in ethanol at different concentrations (1 to 35 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as the standard. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical-scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\textbf{Percentage inhibition} = (A_0 - A_t)/A_0 \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values [14, 15].

RESULTS AND DISCUSSION

The diffusion test was applied to the four above mentioned. The results summarized in **Table 1** showed that the crude extract from *Thymelaea microphylla* Coss. and Dur. prevented the growth of all the tested microorganisms and it has been revealed that the medium diameter of inhibition zone increases proportionally with the increase of extract concentrations.

The obtained inhibition zone varied from 7.00 mm to 30.50 mm with a highest value recorded with *Staphylococcus blanc ATCC 27853 followed by a good inhibition effect with Staphylococcus aureus* ATCC25923 (26.25 mm at 8mg/ml) . Nevertheless the fungus *Aspergillus niger* displayed a small inhibition diameter even with high concentration of 4000 µg/ml in comparison with the other strains. No activity was recorded against *E. Coli atcc 25922, Staphylococcus aureus* ATCC25923*and Aspergillus niger* at low concentration (250 µg/ml and 500 µg/ml).

Table 1. Antimicrobial activity of *Thymelaea microphylla* Coss. and Dur. extract

Bacteria Strains	250µg/ml	500µg/ml	1000µg/ml	2000µg/ml	4000µg/ml	8000µg/ml
E. Coli atcc 25922	-	6.75± 0.57	7.25±0.86	11±01.47	17.75±02.1	23±00
Staphylococcus blanc ATCC 27853	7±01.47	17.5±1.15	18.50±01.5	18.75±02.1	27.5±01.47	**30.5±1.15**
Staphylococcus aureus ATCC25923	-	-	10.25±00	16.5±0.81	18 ±1.75	26.25±1.15
Fungus : Aspergillus niger	-	-	-	-	10±01.47	16.75±00

Antioxidant activity

The analysis of DPPH scavenging activity (SC%) are presented in Figure 1. The crude extract at different concentrations 10^{-1}M,10^{-2}M,10^{-3}M,10^{-4}M. Vitamin C was used as standard exhibited varying degrees of scavenging capacities (concentration-dependent DPPH radical scavenging activity). In the highest concentration a crude extract exhibit 72.0 % in comparison of Vitamin C 64%. Many studies have pointed out that there is a positive correlation between antioxidant activity potential and amount of phenolic compounds of the extracts [16–18]. Therefore, *Thymelaea microphylla* Coss. and Dur. rich in phenolic compounds determined in other study [18].

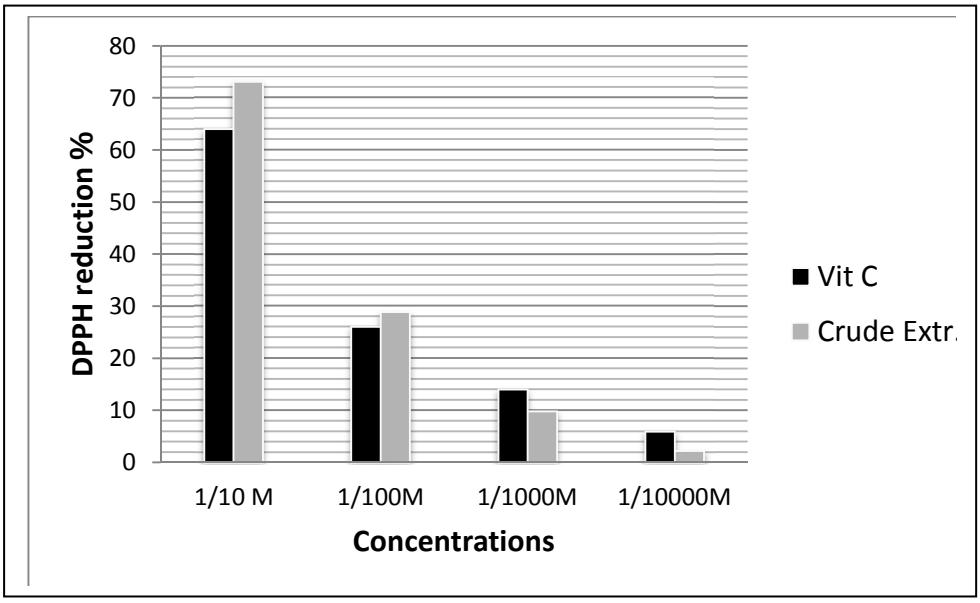

Fig 1. Antioxidant activity of *Thymelaea microphylla* extract

CONCLUSION

This study was designed to evaluate the antioxidant and antimicrobial activities of crude extract from *Thymelaea microphylla* . the results exhibited higher antioxidant activity, which are stronger than the positive control vitamin C. An additional characteristics of *Thymelaea microphylla* extract was its prominent antibacterial activity against a pathogenic microorganism *Staphylococcus blanc ATCC 27853.* The results presented here can be considered as the first information on the antimicrobial and antioxydant properties of *Thymelaea microphylla* further work is still needed to identify and characterize their compounds effect.

Acknowledgments

The authors are grateful for the partial financial support of the Ministère de l'Enseignement Supérieur et de la Recherche Scientifique (MESRES - Alger, Algeria).

REFERENCES

[1] L.S. Lai; S.T. Chou; W.W. Chao; *J. Agri. Food Chem*, **2001**, 49, 963.
[2] Y. Arab, A. Zellagui, S. Boutellaa, K. Mesbah and N. Gherraf . *Der Pharmacia Lettre*, **2014**, 6 (4), 522-525
[3] G.J. Koomen, T. Den Blaauwen, K.J. Hellingwerf, R. Ungaro, S. Mobashery. Fighting microbial resistance through deve- lopment of new antimicrobial agents, directed against new specific targets. IUPAC Project., **2002**, -030-1-300.
[4] M. C. Baratto, M.Tattini, , C. Galardi, P. Pinelli, A. Romani, F. Visiolid, et al *Free Radical Research* , **2003**. 37(4), 405–412.
[5] J.Whysner, C. X.Wang, E.Zang, , M. J. Iatropoulos, G. M. Williams, *Food and Chemical Toxicology*, **1994**, 32, 215–222.

[6] P.Quezel, S.Santa, Nouvelle Flore de l'Algérie et des ré gions dé sertiques mé ridionales, vols., **1963**, 1–2. Ed. CNRS, Paris, France.
[7] A. Zellagui, N. Gherraf and S. Rhouati, *Bio Organic and Medicinal Chemistry Letters,* **2012**, 2, 31.
[8] L. S. Noamane, A. Zellagui, K. Mesbah, N. Gherraf, M. Lahouel and R. Salah, Der *Pharmacia Lettre*, **2010**, **2**(5), 428-431.
[9] N. Douhou, K. Yamni, A. Badoc, A. Doura,. *Bull. Soc. Pharm*, **2004**, 143, 31-38.
[10] N. Amari, M. Bouzouina, A. Berkani, B. Lotmani. *Asian Pac J Trop Dis*, **2014**, 4(2), 104-109
[11] A.A. Ahmed, M F. Hegazy, A. Zellagui, S. Rhouati, T.A. Mohamed, A. A. Sayed, M. A. Abdella, S. Ohta , T. Hirata, *Phytochemistry.*, (**2007**), 68, 680–686.
[12] National Committee for Clinical Laboratory Standards [NCCLS] Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically; approved standard M7-A6, (**2003**), 6th ed., Wayne.
[13] M.S. Blois,. *Nature*, (**1958**), **26**, 1199-1200.
[14] T. Masuda, S. Yonemory, Y. Ouyama; Y. Takeda, T. Tanaka and T. Andoh .*Journal of agriculture and food chemistry*, **1999**,47 :1749-1754.
[15] F. Hayase, H. Kato, *J. Nutr. Sci. Vitaminol.*, **1984**, *30*, 37–46.
[16] V.V. Kedage, J.C.Tilak, G.B. Dixit, T.P.Devasagayam, M. Mhatre, *Crit. Rev. Food Sci. Nutr.*, **2007**, *47*,175–185.
[17] C. Sarikurkcu, B. Tepe, D. Daferera, M. Polissiou, M. Harmandar, *Bioresource Technol.*, **2008**, *99*, 4239–4246.
[18] T. Mekhelfi, K. Kerbab, G. Guella, L. Zaiter, S. Benayache and F. Benayache. *Der Pharmacia Lettre*, **2014**, 6 (1):152-156.