Biochemical Composition and Biological Activities Assessment of *Periploca laevigata* and *Thymus algeriensis* Leaves Extracts

Hamza Gadhoumi[1,2,*], Moufida Saidani Tounsi[2] and El Akrem Hayouni[2]

[1]*Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis, Tunisia*

[2]*Laboratory of Aromatic and Medicinal Plants, Center of Biotechnology at the Ecopark of Borj-cédria, BP-901, 2050 Hammam-Lif, Tunisia*

Abstract: The plant traditional medical application indicates great antioxidant and antimicrobial properties. The present study investigated the Biochemical and antimicrobial and antifungal activity of *Periploca laevigata* and *Thymus algeriensis* leaves extracts. The biochemical (total phenolic content, total flavonoids, and condensed tannins) and biological activity (antioxidant potentials, and antimicrobial activities) of leaf extracts of *Periploca laevigata* and *Thymus algeriensis* were assessed. The results showed that the leaves of the *Thymus algeriensis* extract has the highest levels of polyphenols, flavonoids, and tannins 120 ± 2.02 mg GAE/g DR, 46 ± 2.5 mg RE/g DR, and 22± 2 mg CE/g DR, respectively. The GC-MS analyses showed that the plants have interesting volatile compounds. Therefore, leaves of *Thymus algeriensis* extract possess high anti-oxidant activities (total antioxidant capacity, 2,2-diphenyl-1-picrylhydrazyl and ferric reducing antioxidant power scavenging activities). In addition, this extract exhibited high antifungal and antimicrobial activities, especially toward Gram-positive bacteria. This results highlights 'importance as dietary sources for natural antioxidants and antimicrobial can be used in traditional medicine and the pharmaceutical industry.

Keywords: Medicinal plants, *Periploca laevigata*, *Thymus algeriensis*, Antioxidant, Antimicrobial activity, Antifungal activity.

INTRODUCTION

Nowadays, there is growing interest in new compounds that inhibit growth of filamentous fungi. The most attractive are the ones of low toxicity and high biodegradability or the ones of high selectivity of action, not affecting the growth of saprophytic fungi. *Phytophtora infestans* causes serious losses of potato crops worldwide and is probably the most important pathogen of potato and tomato today. Late blight of potato is identified by black/brown lesions on leaves and stems that may be small at first and appear water-soaked or have chlorotic borders, but soon expand rapidly and become necrotic. In humid conditions, *P. infestans* produces sporangia and sporangiophores on the surface of infected tissue. Potato tubers become infected in the field when sporangia are washed from the foliage into the soil. Infections generally begin in tuber cracks, eyes or lenticels [1]. *P. infestans* is an oomycete protist. *P. infestans* was originally thought to be a fungal species due to its filamentous structure and metabolic strategie1. *Phytophthora* have two kinds of resting or survival spores that they produce, both of which have thick cell walls that allow them to survive in suboptimal environments for long time periods. The two kinds of spores are oospores, formed from sexual recombination and chlamydospores, asexual survival spores [2, 3]. More and more human and plant pathogenic fungi develop resistance against currently used many compounds and therefore do not respond to antimicrobial and antifungal treatments. Medicinal plants have always been used to prevent or treat various diseases. Plants constitute a natural source of a diverse array of antioxidant molecules that have the potential to eliminate or neutralize the deleterious Reactive oxygen species (ROS) [3]. The *Periploca laevigata* that belongs to the genus *Periploca* (Asclepiadaceae), is habitually grown in non-agriculturally viable locations. *Periploca laevigata* has a wide native distribution different region such as in Spain, Morocco, Algeria, Tunisia, Libya, Egypt, Malta and Sicily [4]. In Tunisia *P. laevigata* extends from Jbel Ichkeul in the North where the rainfall ranging between 300 and 600 mm/year, where it forms small spots, to the Saharan borders where the rainfall is about 100 mm/year [5, 6]. Recently, several studies reported the antioxidant, antimicrobial, antimalarial and hepatoprotective effects of some Periploca species; say *P. aphylla*, *P. linearifolia* and *P. angustifolia* [7-9]. In addition, several studies reported that T. algeriensis extracts contained interesting Bioactive compound which proves their biological activities (*in vitro* and *in vivo* activity) [10-13]. The aim of this study was to explore the phytochemical composition of *Periploca laevigata* and *Thymus algeriensis* leaves extracts and characterization of volatile compounds and investigation of biological activity namely antioxidant

*Address correspondence to this author at the Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis, Tunisia;
E-mail: gadhoumih@gmail.com

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potency and their effect on the antimicrobial and antifungal activities.

MATERIALS AND METHODS

Materials

The medicinal plants *Periploca laevigata*, and *Thymus algeriensis* collected from Orbata National Park Mountain (Gafsa, Tunisia) with coordinates: N 34° 22′49.8″and E 9° 3′23.4″, and extracted (methanol extract) using the method described by [14]. The Bacteria strains (*Escherichia coli 8739 ATCC, Staphylococcus aureus 25923 ATCC, Enterococcus aerogenes ATCC (13048), Enterococcus faecalis 29212 ATCC, Pseudomonas aeruginosa 27853 ATCC, Pseudomonas aeruginosa DSM1128)* and the fungi (*Fusarium oxysporum, Fusarium graminearum, Aspergillus niger, Phytophtora infestans*) were obtained from Laboratory from Extremophile plants, Center of Biotechnology at the Ecopark of Borj-cédria. Hammam-Lif. Tunisia.

Determination of Total Phenolic Content, Total Flavonoid Content, and Tannins Contents

The total polyphenols phenolic content was determined according to the method [15].

The flavonoids content was determined based on the capacity of the formation of a yellow flavonoid-aluminum complex whose maximum observance is at 510 nM [16]. The determination of the condensed tannins in each extract using the method described by [17], modified by [18]. The amount expressed as Gallic acid equivalents (mg GAE/g DR), as routine equivalents (mg RE/g DR), as catechin equivalent per gram of extract (mg CE/g DR), respectively.

Headspace Solid-Phase Microextraction of Volatile Compounds

The volatile compounds analyzed by Headspace Solid-Phase Microextraction (HS-SPME) coupled with Gas Chromatography-Mass Spectrometry (GC-MS) as previously described [19]. The GC-MS headspace analysis was performed with an iron source's temperature of 240°C and an ionization voltage of 70eV. The mass spectrometer was operated in scan mode from m/z 50 to 350. Peak areas were determined for each compound by integrating a selected ion unique to that compound. The volatile compounds were identified by matching their mass spectra with those in the NIST1. I Library of MS spectra. The Kovats retention index (RI) was calculated with a homologous series of n-alkanes (C6-C28) under the same conditions applied for the sample analyses.

Antioxidant Activity

The antioxidant activities were measured by different tests; the scavenging activity on DPPH radical of extracts was estimated as reported by [20]. DPPH scavenging activity was presented with IC50 values, defined as the concentration of the sample required to scavenge 50% of free radicals present in the test solution.

The reducing power was determined according to the method reported by [21]. The intensity of the Bluegreen appearing color was measured at 700 nm on UV–VIS spectrophotometer. The amount of sample providing 0.5 of absorbance, Vitamin C was used as the control.

The total antioxidant capacities of each extract were estimated using the method described by [22]. 0.1 ml of extract was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mm sodium phosphate and 4 mm ammonium molybdate). After incubation in boiling water bath for 90 min, the absorbance was measured at 695 nm.

Antifungal and Antimicrobial Activity

The antifungal and antimicrobial activity of the extracts was determined by the diffusion method in agar medium cited by [23, 24] with a slight modification, this method was employed to determine the inhibition diameter of the extract against 6 strains of bacteria and 4 fungi.

RESULTS AND DISCUSSION

Total Phenolics, Total Flavanoids and Condensed Tannins Content

Polyphenols constitute one of the most preponderate groups of substances in plants, including a wide variety of bioactive molecules [20]. Phytochemical analysis revealed that the amount of total phenolic, flavonoids and condensed tannins varied in the plants. In this study *Thymus algeriensis* leaves extracts contained high levels of polyphenols, confirming there highest antioxidant activity to compare with *Periploca laevigata.* The results indicated in Table **1** showed that the *Thymus algeriensis* leaves extracts contained highest levels of polyphenols, total

Table 1: Biochemical Composition (Total Phenolic Content, Total Flavonoids, and Condensed Tannins), and Antioxidant Potentials of *Periploca laevigata*, and *Thymus algeriensis*

Extracts	Total phenolics (mg GAE/g DR*)	Flavonoids (mg RE/g DR**)	Condensed tannins (mg CE/g DR***)	Total antioxidant capacity (mg GAE/g DR)	DPPH (IC_{50}; µg/ml)	Reducing power (IC_{50} µg / ml)
thymus algeriensis	120 ± 2.02	46 ± 2.5	22± 2	72.5± 2	25.1±3.5	15.3± 2
Periploca laevigata	83 ± 3	36 ± 1.5	20± 1.5	51.3 ±4.5	34.5± 3.2	28.2± 1.5
Vit C				- - -	5.2± 1.98	12.5± 1.5
AG				12.1 ±1.2	- - -	- - -

Results are expressed as mean of 3 experiments ± SD.
*mg GAE/g DR: mg gallic acid equivalents per g dry residue.
**mg RE/g DR: mg of rutin equivalent per gram dry residue.
***mg CE/g DR: mg catechin equivalent per gram dry residue.
Vit C: Vitamin C.
AG: Gallic acid.

flavanoids and condensed tannin content: 120 mg GAE/g DR, 46 mg RE/g DR, and 22 mg TA/g DR, respectively, to compared with *Periploca laevigata* leaves extracts: 83 mg GAE/g DR, 36 mg RE/g DR, and 22 mg TA/g DR, respectively. Our results correlate with those discovered by several authors [10-13] which they showed high phytochemical content.

The extraction yield and the content of bioactive compounds including polyphenols, flavonoids, and tannin are influenced by the nature of plants raw materials [22].

Volatile Compounds

Nine major volatile compounds were identified with remarkable differences between the plants (Table **2**). The results showed that many compounds were detected only from *Thymus algeriensis* extract was 3,9-Epoxy-1-p-menthene, other compounds were detected only from *Periploca laevigata* extract were: l-Phellandrene, and 2-Bornanone. The other compounds were detected in two the extracts (α-pinene, Camphene, α-Terpinene, 1,8-Cineole, Fenchone, Terpinen-4-ol). Several studies have been published on the composition of the volatile constituents of the plants using different methods confirming the presence of his volatile compounds [10-13]. The volatile compounds might be influenced by the source of the plant material, genetic and environmental factors [12, 13]. These results suggest that the aromatic compounds from plant raw materials could give positive aroma attributes to the flavor plants extract. The aromatic compounds present numerous biological and pharmacological proprieties such as 1,8-cineole possess high antimicrobial activity antimicrobial [12].

Antioxidant Activity

The antioxidant activities of the plants were investigated namely total antioxidant capacity, radical DPPH scavenging activities, and the reducing power

Table 2: Aromatic Compounds Identified from Fermented Beverages Extracts of by GC-MS Head Space

Compounds	RT	% Area *Periploca laevigata*	% Area *Thymus algeriensis*	Chemical formula
α-pinene	5.03	17.3	14.2	$C_{10}H_{16}$
Camphene	7.65	43.8	19.06	$C_{10}H_{16}$
l-Phellandrene	8.63	7.2	NF	$C_{10}H_{16}$
α-Terpinene	8.83	10.5	12.14	$C_{10}H_{16}$
1,8-Cineole	9.06	2.6	42.1	$C_{10}H_{18}O$
Fenchone	10.13	3.01	2.2	$C_{10}H_{16}O$
2-Bornanone	11.9	11	NF	$C_{10}H_{16}O$
Terpinen-4-ol	12.48	4.5	5.2	$C_{10}H_{18}O$
3,9-Epoxy-1-p-menthene	12.6	NF	5.1	$C_{10}H_{16}O$

Results are expressed as mean of 3 experiments.

(Table **1**). Furthermore, the leaves of *Thymus algeriensis* extract process high antioxidant activity 72.5 mg GAE/g DR. The total antioxidant capacity is essentially due to the presence of phenolic and bioactive compounds in the samples. Results relative to the DPPH scavenging activity of the plant extracts showed that the *Thymus algeriensis* extract exhibited highest antioxidant activity (IC_{50} = 25.1 µg BHT/ml), compared to *Periploca laevigata* IC_{50} = 34.5 µg BHT/ml. The reducing power assay expressed as EC50 (µg Vit C/ml), All the samples were able to reduce Fe3+ to Fe2+, but the results differed significantly between plant materials. The leaves of *Thymus algeriensis* extract process highest reducing power 15.3 µg / ml. It's well-known that nutritional value of phenolic compounds is strongly related to their antioxidant activity or their ability to counteract oxidative stress which is expressed by excessive production of reactive oxygen species [25].

Antimicrobial and Antifungal Activities

The antimicrobial Antifungal activities tested by disc diffusion method of obtaining plant extracts presented in Tables **3** and **4**. The present study showed that the different extract exhibited high antimicrobial antifungal activities. Furthermore, the *Thymus algeriensis* extract possesses the highest antimicrobial and antifungal activities to compare the *Periploca laevigata* extract. The high antimicrobial and antifungal activities confirmed that the richness of leaves extracts by bioactive compounds. These results show a high correlation between polyphenol content and antimicrobial activity, especially toward gram-positive bacteria: *Staphylococcus aureus 25923 ATCC and Enterococcus faecalis 29212 ATCC*. This data highlights plants 'importance as dietary sources for natural antioxidants can be used in traditional medicine and the pharmaceutical industry. The volatile compounds detected in these plants have high antimicrobial and antifungal activities. Against fungi, the terpene phenols in extract cause several damages such as morphological disturbances of mycelial hyphae, rupture of the plasma membrane and alteration of the structure of mitochondria [25, 26]. Strong correlations between total phenolic compounds content, total flavonoid amount, volatile compounds and radical scavenging activity and antifungal activity were also observed. This shows that the two plants can constitute an important reserve interesting metabolites, whose active principles can be used in several fields

Table 3: Antimicrobial Activity of Leaves Extract from *Periploca laevigata and Thymus algeriensis*

Bacteria	Gram	Inhibition zone (mm)		
		Thymus algeriensis	***Periploca laevigata***	**Streptomycin**
Escherichia coli 8739 ATCC	Gram (-)	8± 3	6.7± 2	15 ± 2
Staphylococcus aureus 25923 ATCC	Gram (+)	12.5± 1	9± 2	18.5 ± 2
Enterococcus aerogenes ATCC (13048)	Gram(-)	10.5± 2	7.5± 2	25.5± 1
Enterococcus faecalis 29212 ATCC	Gram (+)	15.5± 4	10± 2	20.5 ± 3
pseudomonas aeruginosa 27853 ATCC	Gram (-)	7.5± 1	9.2± 1	16.5 ± 2
pseudomonas aeruginosa DSM1128	Gram(-)	4.2±0.5	8.5±2	10.5±3

Results are expressed as mean of 3 experiments ± SD.

Table 4: Antifungal Activity of Leaves of *Leaves extract* from *Periploca laevigata* and *Thymus algeriensis*

Strains	***Thymus algeriensis***	***Periploca laevigata***	**Antifungal (control)**
Fusarium oxysporum	4.5 ± 1	8 ± 2	12 ± 2
Fusarium graminearum	12 ± 1.5	11 ± 2	11 ± 3
Aspergillus niger	10.5 ± 1	9 ± 1.5	15 ± 1.5
Phytophtora infestans	7 ± 1	6.5 ± 3	12 ± 1.5

Results are expressed as mean of 3 experiments ± SD.

such as the agro alimentary industries, pharmaceutical and agronomy.

CONCLUSION

The results showed that the leaves of the *thymus algeriensis* extract contained high levels of polyphenols, flavonoids, and tannins, and the interesting volatile compounds. *Periploca laevigata* and *thymus algeriensis* a leaf extracts were shown to possess both *in vitro* and *in vivo* antioxidant and antimicrobial Antifungal properties. These results show a high correlation between polyphenol content and antimicrobial and antifungal activities. *Periploca laevigata* and *thymus algeriensis* represent good candidates for consumption as health wellbeing.

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