Evaluation of the antioxidant and antibacterial activities of Tunisian *Artemisia Herba-alba* essential oil

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Abstract

Tunisia is characterized by a climate that allows the proliferation of many plants rich in active substances with multiple biological activities and can replace the use of antioxidants and synthetic antibiotics. For this reason, *Artemisia* leaves and twigs were collected from the central region of Tunisia (Thala). The essential oil was extracted using hydrodistillation and analyzed using GC/MS, the antioxidant activity of *Artemisia Herba-alba* was evaluated by the DPPH test and the antibacterial powers against four bacterial strains was measured by the agar well method diffusion. GC/MS results showed that the main components of *Artemisia Herba-alba* essential oil were β-thujone (23.9 %) and chrysanthenone (17.4 %). Indeed, the results showed a potent antioxidant effect (85.2% inhibition of free radicals DPPH) and the IC50 value was 84.8 µg/ml. Concerning the antibacterial activity, the oil was active against Gram-negative and Gram-positive bacteria. A strong effect was observed against *Salmonella* (29 mm) and *Bacillus* (22.5 mm). To conclude, the antioxidant power and the antibacterial activity are strongly correlated with the chemical composition of the essential oil.

Keywords: *Artemisia Herba-alba*, essential oil, antioxidant activity, antibacterial activity

INTRODUCTION

During the last decades, a concern about the undesirable effects of synthetic molecules intended for the fight against oxidative stress and bacterial infections. It therefore seems important to find an alternative to the use of synthetic antioxidants and conventional antibiotics (Rosenthal *et al.*, 2012). Medicinal plants are considered as an essential raw material source for the discovery of new molecules necessary for the development of future drugs, without side effects (Benbelaïd *et al.*, 2014). An example of these metabolites are essential oils and plant extracts (Rahman and Sattar, 2011). The genus Artemisia belongs to the Asteraceae family, Artemisia species are widely used in traditional medicine. Over 300 species of this genus are found mainly in arid and semi-arid areas of Europe, America, North Africa and Asia (Nikolova *et al.*, 2010). The development of new antioxidants and antibiotics of better quality and of lower toxicity is essential. For this purpose, the investigation of plants represents an invaluable potential (Adams and Gmünder, 2007).

In this context, this study aims to evaluate the antioxidant activity of *Artemisia Herba alba* the essential oil collected from the region of Thala (Tunisia) by the reducing power and the inhibition of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), as well as its antibacterial activity against gram positive and gram negative strains.

MATERIAL AND METHODS

Plant material

The aerial part (Leaf, Stem) of *Artemisia Herba-alba* were collected from the region of Thala (Tunisia) (latitude 35° 35.583' N, longitude 008° 39.717' E, altitude 847 m, semi-arid bioclimate).

Essential oil extraction

The extraction of essential oils was carried out by steam distillation, this method consists in placing the plant material on a grid located a few centimeters from the bottom of the extractor filled with water. The steam enriched with volatile constituents is condensed under the effect of a cooling system. The floral water is collected in a glass balloon and the separated essential oil is collected in an opaque glass bottle. The extraction was carried out for four hours at a temperature of 100 °C.

Essential oil gas chromatography analysis

The essential oil obtained is diluted with n-pentane (Hosni *et al.*,2008), then analyzed by high resolution gas chromatography using an Agilent 6890 brand device (Agilent Technologies España, SL, Las Rozas, Spain) equipped with a flame ionization detector (FID), an auto-injector, an autosampler and an Rtx-1 non-polar column (30 m* 0.32 mm, film thickness 0.25 µm). The system is controlled by GC-Solution type software which ensures the electronic integration of the different peaks.

Identification of volatile compounds of essential oil

The volatile compounds of essential oils were identified by calculating their retention index (IR) from a range of linear alkanes (C8-C25) injected under the same analytical conditions (Van Den Dool and Kratz, 1963). The calculation of the retention indices for volatile compounds is given by the following equation:

$$RI = [n + (TR_i - TR_n) / (TR_{(n+1)} - TR_n)] * 100$$

RI: unidentified peak retention index, **n**: number of carbon atoms of the aliphatic hydrocarbon eluted just before the peak atidentify,$\mathbf{RT_i}$**:** retention time of the unidentified peak, $\mathbf{RT_n}$**:** retention time of the aliphatic hydrocarbon eluted just before the peak to be identified, $\mathbf{RT_{n+1}}$**:** retention time of the aliphatic hydrocarbon eluted just after the peak to be identified

1, 1-Diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity

The antioxidant capacity of *Artemisia Herba Alba* essential oilwas evaluated according to the method described by (Braca *et al.*, 2002). The diluted essential oil solutions were prepared in methanol. Ascorbic acid was used as the standard. Then 1 mL of 0.004% DPPH solution was mixed with 1 mL of sample solution, the mixture was then shaken and kept in the dark for 30 min and optical density was measured at 517 nm. The radical-scavenging activities, expressed as percentage inhibition of DPPH, were calculated according to the following equation:

$$\mathbf{I\ (\%) = [(A0\text{-}A1)/A0]\ x\ 100}$$

I: DPPH inhibition (%), **A0**: absorbance of the blank, **A1**: absorbance of the extract/standard.

The concentration of sample required for 50% inhibition was determined and represented as IC_{50} for each of test solution, which is expressed as µg/ml. All measurements were performed in triplicate.

Antibacterial activity

The antibacterial activity of the essential oil was measured by means of the agar well diffusion test described by Güven *et al.*(2006) against four bacterial strains (Listeria monocytogenes (foodstuff 2132), *Bacillus subtilis* ATCC 6633, Escherichia coli ATCC 25922, Salmonella enterica (foodstuff)). A suspension (100 µl) of bacteria was spread on the plates of nutrient agar, then 6 mm wells were bored using a sterile cork borer. 60 µl of essential oil were placed into the wells. Finally, they were incubated at 37°C for 48 h and the antibacterial activity was evaluated by measuring zone of inhibition. The tests were carried out in triplicate.

Statistical analysis

All data were subjected to statistical analysis by the variance according to the GLM procedure of the software SAS 2002 and compared by Duncan multiple rank test (Duncan, 1955). The model used was:

$$\mathbf{Yij = \mu + Ai + Eijk}$$

Where**Yij**: dependent variable. **µ:** overall of Y; **Ai:** effect of the ith essential oil; **Eijk:** residual error.

RESULTS AND DISCUSSION

Chemical composition

30 compounds of *Artemisia Herba alba* essential oil are identified and listed in table 1, representing 99.5% of the oil. The chemical class distributions of the essential oil could be separated into five classes, Monoterpenes represented about 94.9 % of the total constituents of essential oils. Oxygenated monoterpenes constituted the main chemical class of the oil (74.2 %) and they were represented by 9 derivatives, with β-thujone (23.9 %), chrysanthenone (17.4 %), α-thujone (10.3 %) and 1,8-cineole

Table 1: Chemical composition (% total peak area) of Artemisia Herba-alba essential oil

N°	Compound	RI[a]	Composition (%)
1	α-thujene	932	-
2	α-pinene	939	3.25
3	sabinene	964	3.31
4	β-pinene	983	1.39
5	β-myrcene	992	2.34
6	α-phellandrene	998	0.12
7	3-Carene	1006	0.14
8	***p*-cymene**	***1015***	***8.27***
9	α-terpinene	1018	0.14
10	trans-β-Ocimene	1038	0.99
11	γ-terpinene	1062	0.38
12	α-terpinolene	1076	0.37
13	*1,8-cineole*	*1033*	*9.78*
14	linalool	1101	1.48
15	**β-*thujone***	***1103***	***23.92***
16	camphor	1145	-
17	β-terpineol	1146	-
18	terpinen-4-ol	1164	5.23
19	*p*-menth-1-en-8-ol	1183	4.7
20	*cryptone*	*1189*	-
21	**α-*thujone***	***1194***	***10.34***
22	*p*-cymen-8-ol	1196	-
23	**chrysanthenone**	**1253**	***17.4***
24	geraniol	1255	-
25	*cis*-chrysanthenylacetate	1263	0.12
27	Nerylacetate	1356	1.28
26	β-cubebene	1349	0.05
28	β-elemene	1389	0.57
29	β-caryophyllene	1420	1.2
30	β-farnesene	1453	0.11
31	α-humulene	1456	0.09
32	germacrene-D	1477	1.02
33	α-amorphene	1504	0.06
34	elemol	1547	0.55
35	ledol	1563	0.22
36	caryophylleneoxide	1569	0.73
37	*Spathulenol*	*1576*	-
38	globulol	1603	-
39	γ-*eudesmol*	*1629*	-
40	*n*-Nonadecanoicacid	2220	-
	Total identified		**99.55**
	Oxygenated monoterpenes		**74.25**
	Monoterpene hydrocarbons		**20.7**
	Sesquiterpene hydrocarbons		**3.1**
	Oxygenated sesquiterpenes		**1.5**
	Miscellaneous		**0**

RI: Retention Indice on HP-5 column; Values in bold indicate the main components; -: not detected

(9.78 %) as main components. In addition, monoterpene hydrocarbons were represented by 11 compounds representing 20.7 % of all oil. Among these compounds, p-cymene (8.27 %), α-pinene (3.25 %) and Sabinene (3.31 %) were the most important. Sesquiterpenes constituted 4.6 % of all oil, among them oxygenated derivatives (3 compounds) represented only 1.5% while hydrocarbons (7 compounds) reached 3.1% of all oil.

The chemical variability of *Artemisia Herba alba* observed in the Tunisian areas (north-west, center and south-east of Tunisia) showed various compositions dominated either by a single component (α-thujone, camphor, chrysanthenone or trans-sabinylacetate)) or characterized by the presence of two or more of these compounds at appreciable levels (Boukrich *et al.*, 2010). Indeed, in our study thujones (α-thujone and β- thujone) present the highest percentage 34.26% of all oil, as reported by Haouari and Ferchichi (2009) thujone are the components which give the specific odor and taste to *Artemisia Herba-alba* plants.

For further comparison, Chrysanthenone reached a high percentage (17.4%), according to (Neffati *et al.*, 2008; Akrout, 2004) this component was not found in the Tunisian *Artemisia Herba alba* essential oil at such high concentrations.

Antioxidant activity of *Artemisia Herba-alba* essential oil

The results reported in Figure 1 showed that for ach concentration, the antioxidant activity of *Artemisia Herba alba* is lower than ascorbic acid which is known by its high antioxidant potential. At a concentration of about 300 µg/mL, the exhibited greatest inhibitory activity reaches as high as 85.2 %. The IC_{50} value obtained for scavenging activity on DPPH radical were evaluated (IC_{50}=84.8 µg/ml) and showed that the oil possessed a potent antioxidant effect (Table 2). from the results reported in Table 1, the property of *Artemisia Herba alba* of being a radical scavenging agent was attributed to its richness in oxygenated monoterpenens (Kadri et al., 2011)or to the synergistic effect of more than one oil compound (Younsi *et al.*, 2016).

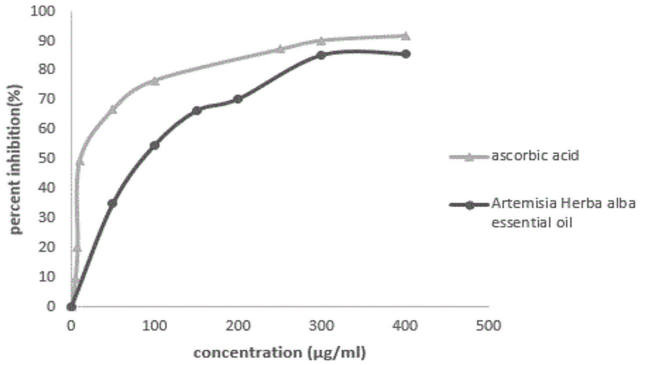

Figure 1: Free radical-scavenging activity of Artemisia Herba-alba essential oil and ascorbic acid on 2,2-diphenyl-1- picrylhydrazyl (DPPH)

Table 2: Inhibition concentration of 50% of free radicals DPPH of *Artemisia Herba-alba* essential oil and ascorbic acid

	IC_{50}(µg/ml)
Artemisia Herba alba essential oil	84.8 [a] ± 0.49
Ascorbic acid	26.8 [b] ±0.1
p>f	<0.0001

IC_{50}: inhibition concentration of 50% of free radicals DPPH. The letters a et b on the same line indicate significant differences at p <0.05

Disagreeing with our current data, (Akrout *et al.*,2010) reported that the essential oil of leaves of *Artemisia Herba-alba* growing in the wild in southern Tunisia exhibited low anti-free radical activity in the method (DPPH). However, the same result was found for different essential oils of aerial parts of *Artemisa Herba-alba* cultivated in southern Tunisia (Mighri *et al.*, 2010).

Antibacterial activity of *Artemisia Herba-alba* essential oil

The *in vitro* antibacterial activity of *Artemisia Herba Alba* was qualitatively and quantitatively assessed by the diameter of inhibition zone, MIC and MBC values.

The data indicated in table 3 that the oil was active against Gram negative bacteria and Gram-positive bacteria. An important effect was noticed against *Salmonella enterica* and *Bacillus subtilis* ATCC 6633 which were very sensitive with an inhibition diameter of 29 and 22.5 mm respectively. Furthermore, *Escherichia coli* ATCC 25922 and *Listeria monocytogenes* were tightly less sensitive with an inhibition zone of 21.3 and 18 mm.

The results in table 4 show that the oils exhibited varying levels of antibacterial activity against the investigated

Table 3: Diameter of inhibition zone in (mm) of *Artemisia Herba-alba* essential oil against four bacterial strains

	Bacterial strains	Inhibition zone diameter in (mm)	
		***Artemisia Herba-alba* essential oil**	**Gentamicine**
Gram +	***Listeria monocytogenes***	18[c] ± 0.3	33.1 ± 0.03
	***Bacillus subtilis* ATCC 6633**	22.5[b] ± 0.15	31.5 ± 0.86
Gram -	***Escherichia coli* ATCC 25922**	21.3[c] ± 0.04	34.1 ± 0.10
	Salmonella enterica	29.0[a] ± 0.14	30.0 ± 0.25
Bacteria effect		<0.0001	

The letters (a - c) on the same line indicate significant differences at p <0.05

Table 4: Minimum inhibitory (MIC) and minimum bactericidal concentration (MBC) of *Artemisia Herba-alba* essential oil

	Bacterial strains	Essential oil of *Artemisia Herba-alba*		
		MIC (µl/ml)	**MBC (µl/ml)**	**MBC/ MIC**
Gram +	***Listeria monocytogenes***	10	80	8
	***Bacillus subtilis* ATCC 6633**	10	80	8
Gram -	***Escherichia coli* ATCC 25922**	20	100	5
	Salmonella enterica	10	100	10

***MIC:** Minimum Inhibitory Concentration, **MBC:** Minimum Bactericidal Concentration*

bacteria ($p<0.05$). all bacteria are inhibited at concentrations ranging from 10 µL/mL to 100 µL/mL. Those concentrations are higher than the doses found by Sbayou *et al.*(2014) and Mighri *et al.*(2010). In fact, our essential oil showed a significant bacteriostatic activity as specified by the ratio MBC/MIC of 5 to 10 that is >4 (Pankey *et al.*, 2004). This activity could be related to the amounts of oxygenated mono-and sesquiterpene hydrocarbons (Rahman and Sattar, 2011). The presence of β-thujone as a major compound in *Artemisia Herba alba* may be responsible for its antibacterial activity. Thus, it is difficult to attribute the activity of a complex mixture to a single or particular constituent. Major or trace compounds might give rise to the antimicrobial activity exhibited. Possible synergistic and antagonistic effect of compounds in the oil should also be taken into consideration (Sbayou *et al.*, 2014).

CONCLUSION

The present work has shown that the essential oil of *Artemisia Herba alba* collected from the center of Tunisia is doubted of considerable antioxidant and antibacterial activities, hence the possibility of using them as antioxidants and natural antibiotics. This biological activity is strongly linked to the chemical composition of the essential oil and to the concentration of these this aromatic and medicinal plant on secondary metabolites. Research on this essential oil should be continued to better estimate other potential as anti-inflammatory, anti diabetic and antifungal activity, even use it as supplement in the field of animal feeding.

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