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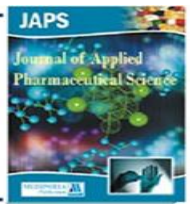

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Chemical Composition, Antioxidant and Antibacterial Activities of Leaves Essential Oil and Ethanolic Extract of Moroccan *Warionia saharae* Benth. & Coss.

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ABSTRACT

The aims of this study were to analyze the chemical composition of leaves essential oil of *Warionia saharae*, to evaluate the antioxidant capacity (DPPH test), antibacterial properties and to investigate toxicity against *Artemia salina* of both oil and ethanolic extract. The results showed that essential oil was toxic (CL50 = 1,56 µg/ml). The GC–MS analysis revealed 52 compounds representing 91.54% of the total oil containing Nerolidol (25,95%) and β-Eudesmol (38,12%) as a major components. The oil (1 mg/ml) exhibited a strong antibacterial effect as a diameter of zones of inhibition (28,5 ± 2,12 and 37,5 ± 3.53 mm) against St. aureus and P. aeruginosa, respectively. While, ethanolic extract (50 mg/ml) exhibited a moderate effect against all tested bacterial strains. MICs values of oil and the extract were ranged 0,031-0,25 µg/ml and 6,25-12,5 mg/ml, respectively. The free radical scavenging activities of the oil was higher than that of ethanolic extract (IC50 = 21,49 and IC50 = 182 µg/ml, respectively).

Keywords: *Warionia saharae*; antioxidant activity; antibacterial activity; CG-MS; ethanolic extract; *Artemia salina.*

INTRODUCTION

In recent years, antibacterial and antioxidant plant properties have gained special interest thanks to their richness of polyphenols which are considered as one of the important phytochemicals having antioxidant properties and having several industrial applications such as in the production of paints, paper and cosmetics, as tanning agents and in the food industry as additives and as natural antibacterial agents. The monotypic genus Warionia was included in the Asteraceae Cichorioideae subfamily by Bremer (1994); and was assigned to Cichorieae tribe by Katinas *et al.*, 2008. *Warionia saharae* Benth. & Coss., is endemic to northwestern edge of the African Sahara desert, in Morocco and in Algeria at Béni-Ounif (South Oran) (Katinas *et al.*, 2008). This species known by the vernacular name of "afessas" or "abessas", is considered to have medicinal properties especially essential oils (Watillon *et al.*, 1987). Decoction of dried leaves is used as antirheumatic, for gastrointestinal disorders and epileptic crisis (Bellakhdar *et al.*, 1986). Crude extracts of the plants showed cytotoxic activities against KB cells cancer (Hilmi *et al.*, 2003).

The chemical composition of *Warionia saharae* essential oil from the leaves was reported by Ramaut *et al.,* (1985); Elamrani *et al.,* (2007) and Znini *et al.,* (2011).

Warionia saharae is a thistle-like aromatic plant, with white latex, and pinnately-partite, somewhat fleshy leaves. The capitula are homogamous with tubular corollas, the anthers are caudate, and the style branches are dorsally covered by acute collecting hairs extending somewhat below the branches bifurcation. The flowering season has been recorded from April to June, while it may extend to July or August if the spring rains are abundant and well spaced (Audissou, 1999).

Essential oils have been widely used in traditional medicine. Among others, antibacterial, antifungal, immunomodulatory, antiinflammatory, and antirheumatic activities have been described (Saller *et al.,* 1995; Hammer *et al.,* 1999; Reichling, 2001). To the best of our knowledge, the biological effects of *W. saharae* essential oil have not been studied yet. The aim of this work is to analyze the chemical composition of essential oil from leaves of *W. saharae* and to investigate its antibacterial activities against some of pathogenic and spoilage bacteria by evaluating minimal inhibitory concentrations (MICs), antioxydante and toxicity effects. Also, we evaluate the same biological activities of ethanolic extract of *W. saharae* leaves.

MATERIALS AND METHODS

Plant material

Aerial parts of *W. saharae* were collected from Tata region (650 Km south of Casablanca) in April 2010. The plant was identified by professors Fougrach Hassan and Mohammed Hsain, and voucher specimen was deposited in herbarium of the biological department of faculty of sciences Ben M'sik, University Hassan II-Mohammadia.

Chemicals

Folin-Ciocalteu Reagent (FCR), Butylated hydroxytoluene (BHT) and α-tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, quercetin and ascorbic acid were obtained from Somaprol (Casablanca-Morocco) and all other chemicals were of analytical grade.

Extraction of essential oil

One hundred grams of air-dried *W. saharae* leaves were cut in small pieces and placed in a round-bottom flask with 0,8 L distilled water, and the essential oil were obtained by hydrodistillation after 3 h in a Clevenger-type apparatus. The collected oil was preserved in a dark sealed vial at 4°C for further analysis.

Preparation of ethanol extract

50 g of powdered leaves of the *W. saharae* was extracted with absolute ethanol at room temperature (2×200 ml) for 24h. Following the filtration, the ethanol extract was evaporated to dryness under reduced pressure at 45°C. The extract yield was 8.90 ± 0.79% (w/w).

Samples extractions for total phenolic and total flavonoïd compounds

Samples of total phenolic and total flavonoïd compounds were extracted from *W. saharae* powder as described by Makkard *et al.,* (1999) slightly modified. The powder sample (1 g) was extracted twice with 10 ml of aqueous methanol solution (50%). The two volumes were combined, made up to 20 ml, centrifuged at 4500 rpm for 20 min and stored at +4°C in the dark for analysis.

Gas chromatography–mass spectrometry (GC–MS)

The chemical composition of the essential oil was analyzed using a gas chromatograph (*Trace GC ULTRA*) equipped with a capillary column (5% phenyl-polymethylsiloxane, 30 m length; 0,25 mm i.d.; 0,25 µm film thickness) and coupled to a mass selective detector (*Polaris Q MS* type *trappe ionique).* Helium was used as the carrier gas at a flow rate 1.4 ml/min. The injector, used in the split mode, and detector temperature were 220 and 300°C respectively, oven temperature programmed from 40 to 300°C at 4°C/min. Isothermal temperature was at 300°C for 20 min. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–350. The ion source temperature was set at 200°C. Identification of components was assigned by matching their mass spectra with the NIST/EPA/NIH Mass Spectral Library Version 2.0 (2002) and comparing their arithmetic retention indices to n-alkanes (C8-C20) in the same conditions, with reference libraries (Adams, 2007) and from the literature. The quantity of all identified components was investigated by using a percent relative peak area as shown in Table 1.

Table. 1: Chemical composition of essential oil obtained by hydrodistillation from Moroccan *W. saharea* collected from Tata region in April 2010.

N°	AI	Compounds	Percentage %
1	1008	β-Ocimene (cis)[a,c]	0,05
2	1045	Sabinene[a,c]	0,08
3	1061	2,3-Dehydro-1,8-cineole[a]	0,04
4	1074	α-Phellandrene[a,c]	0,1
5	1086	α-Terpinene[a,c]	0,09
6	1094	β-Cymene[a]	0,09
7	1098	1,8-Cineole[a,c]	0,42
8	1124	γ-Terpinene[a,c]	0,2
9	1130	Quercetin 7,3',4'-trimethoxy[a]	0,06
10	1148	Terpinolene[a,c]	0,04
11	1159	Linalool acetate[a]	1,73
12	1220	Sabinene hydrate (trans)[a,c]	0,27
13	1231	α-Terpinenyl acetate[a,c]	0,44
14	1371	Copaene[a,b]	0,04
15	1432	α-Santalol (cis)[a]	0,08
16	1446	α-Guaiene[a,b]	0,03
17	1449	Cedren-13-ol, 8-[a]	0,18
18	1459	Naphthalene,1,2-dihydro-4-(4-methylphenyl)-[a]	2,23
19	1466	3-(Hydroxymethyl)-1-phenyl-1-heptadecyn-3-ol[a]	0,05
20	1472	γ-Himachalene[a,b]	0,2
21	1475	α-Longipinene[a]	0,34
22	1486	β-Spathulenol[a]	0,17
23	1491	Thujopsene[a]	0,1
24	1506	Nerolidol (Trans)[a,c]	25,95
25	1514	Caryophyllene oxide[a,c]	0,91

26	1517	Ledene[a]	0,45
27	1525	β-Guaiene[a,b]	0,08
28	1529	Isoledene a	0,58
29	1536	α-Eudesmol[a,c]	0,22
30	1544	Cadinene[a,b,c]	0,15
31	1552	δ-Selinene[a,c]	5,4
32	1565	β-Eudesmol[a,c]	38,12
33	1579	Cubenol[a]	1,04
34	1589	α-Elemene[a]	0,93
35	1601	Ledene oxide-(II)[a]	0,17
36	1613	3-(Hydroxymethyl)-1-phenyl-1-heptadecyn-3-ol[a]	0,08
37	1618	Chamazulène[a]	0,18
38	1631	γ-Costol[a]	0,35
39	1639	Cycloisolongifolene, 8- hydroxy-, endo-[a]	0,13
40	1644	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol[a]	1,46
41	1660	α-Copaen-11-ol[a]	0,2
42	1696	Menthol, 1'-(butin-3-on-1-yl)-, (1R,2S,5R)-[a]	0,12
43	1702	Cycloisolongifolene,9,10-dehydro-[a]	0,42
44	1713	(6Z)-4-isopropylidene-1-methyl-bicyclo[5.3.1]undec-6-en-10-ol[a]	1,37
45	1728	Methyl 3-oxooctadecanoate[a]	0,52
46	1734	Betula camphor[a]	0,08
47	1738	Fucoxanthin[a]	0,04
48	1750	Dibutyl phthalate[a]	0,5
49	1787	Euphorbiafactor Ti5[a]	0,26
50	1798	Stearic acid, 3-(octadecyloxy)propyl ester[a]	0,13
51	1803	Dimethoxyglycerol docosyl ether[a]	0,04
52	1881	Dodecamethyl-1,11-dihydrohexasiloxane[a]	4,63
Total			91,54

N° = The numbering refers to elution order on capillary VB-5 column.
AI = arethmetic retention indices relative to n-alkanes.
a = Comparison of mass spectra data with NIST/EPA/NIH Mass Spectral Library.
b = Comparison mass spectra data with literature data (Adams).
c = comparaison of the compunds identified with literature data.

Dosage of total phenolic compounds

Total phenolic compounds were determined following the method adapted from Muchuweti *et al.,* (2006) slightly modified. To a sample of 100 µl, distilled water was added to make a quantity of 2 ml, followed by addition of 1 ml of FCR (diluted 10 times) and sodium carbonate (20%). After 40 min at room temperature, absorbance at 725 nm was read on a spectrophotometer (Uviline 9400 UV-visible) against a blank that contained methanol instead of sample. Total phenolic compounds were expressed as milligrams of gallic acid equivalents/g dry weight (mg GAE/gDW). Samples were analyzed in three replications.

Determination of total flavonoid compounds

Total flavonoïd compounds were measured according to a colorimetric assay slightly modified (Zhishen *et al.*, 1999). A 250 µl of quercetin standard solution at different concentrations or diluted sample was added to 1 ml of distillate water. At time 0 min, 75 µl of $NaNO_2$ (5%) was added to the mixture. After 5 min 75 µl of $AlCl_3$ (10%) was added. At 6 min 500 µl of NaOH (1N) was added to the mixture. Immediately, the solution was diluted by adding 2,5 ml of distilled water and mixed thoroughly. Absorbance of the mixture was determined at 510 nm against the prepared blank. Total flavonoïd compounds in leaves extract of *W. saharae* was expressed as milligrams quercitin equivalents/g of dry weight (mg QE/gDW). Samples were analyzed in three replications.

DPPH radical scavenging assay

The radical scavenging abilities of *W. saharae* oil and ethanol extract was evaluated according to the slightly modified method of Pothitirat *et al.,* (2009). 0,5 ml of various concentrations of samples in methanol and standards BHT, vitamin C and α-tocopherol separately were added to 1 ml of a 100 µM methanol solution of DPPH. The reaction mixture was incubated in the dark at room temperature for 20 minutes. The optical density was monitored at 517 nm against blank containing methanol. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity as percentage of inhibition (%IP) of DPPH radical:

$$\% IP = \{(Abs_{control} - Abs_{sample})/(Abs_{control})\} \times 100$$

Where; $Abs_{control}$ is the absorbance of DPPH radical + methanol; Abs_{sample} is the absorbance of DPPH radical + sample extract or standard. The antioxidant activity of samples was expressed as IC50 in µg/ml required to inhibit the formation of DPPH radicals by 50%. A low IC50 value represents a high antioxidant activity.

Antimicrobial activity

Disc diffusion method

Anti-bacterial activity of essential oil and ethanolic extract was determined by the disc diffusion method (Collin *et al.,* 1995) against the following bacterial strains: Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), Staphylococcus aureus (ATCC 25923) and Listeria monocytogenes ATCC 19117. Microorganisms were obtained from the culture collection of the Department of Microbiology in the Hospital Ibn Rochd-Casabanca Morocco. Microorganisms were maintained on Muller-Hinton agar (MHA). The inoculums suspension were prepared by diluting suitably overnight (24 h at 37°C) cultures in Muller Hinton Broth medium (BMH) with sterilized distilled water. The cell density was standardized with spectrophotometer (620 nm) to contain $1\text{-}3\times10^8$ microorganisms CFU/ml. The inoculum (100 µl) containing 10^6 CFU/ml of each bacterial strain was swabbed on the entire surface of MHA. Sterile buvard paper discs (6 mm in diameter) were impregnated with 10 µl of oil, extracts and their dilutions and then placed on the surface of inoculated Petri dishes (90 mm). The plates were left at ambient temperature for 30 min to allow excess prediffusion of extracts prior to incubation at 37°C for 24 h. Diameters of inhibition zones were measured (mm). Each experiment was done in duplicate. Standard disc of Ampicillin (30 µg) and blank discs (impregnated with DMSO) are used as positive and negative controls, respectively. The MIC was determined as the lowest concentration of extracts inhibiting visible growth of each organism on the MHA.

Macrobroth dilution test

The MIC of essential oil was determined by a two-fold dilution method in DMSO. A fixed volume (3ml) of BMH medium was distributed in test tubes and inoculated with inoculums (30 µl) containing 10^6 CFU/ml of previously bacterial

strains. Ampicilin and DMSO are used as control positive and negative respectively. Tubes were incubated for 24 h at 37°C. The lowest concentration of essential oil that completely inhibits visual growth of bacteria (no turbidity) is recorded as MIC.

Acute toxicity assay toward *Artemia salina*

Toxicity to *A. salina* (brine shrimp) larvae was determined as described by Pimenta *et al.,* (2003) and Meyer *et al.,* (1982). The larval mortality was recorded at 24 h. The lethal concentration (LC50) is the amount of a substance that causes the death of 50% of a group of test animals. The LC50 value provides an indication of the short-term poisoning potential of extracts. *A. salina* encysted eggs (10 mg) were incubated in 100 ml of artificial seawater (salinity 35 g/l) under artificial light at 25°C. After incubation for 24h, nauplii were collected with a Pasteur pipette and kept for an additional 24h under the same conditions to reach the metanauplii stage. Briefly, brine shrimp were exposed to potential toxic extracts in 24-well cell culture plates. Essential oil and ethanolic extract were dissolved separately in DMSO and diluted to decrease concentrations. About 10 nauplii were added to each well with oil and ethanolic extract. Well control negative and positive containing respectively DMSO and seawater. The number of dead brine shrimp was recorded after incubation at 25 °C for 24 h and the LC50 was calculated from the regression curve between the percentage mortality versus the logarithm of treatment concentration in Microsoft Excel. Tests were performed in three replicates for each concentration.

Statistical analysis

Data are expressed as mean Standard Deviation (SD). The statistical analysis was performed using the software Microsoft Excel 2007. The IC50 was obtained with a computer program GraphPad (Prism V. 5.00) by plotting the percentage of inhibition versus the logarithm concentrations. Each determination was performed in triplicate.

RESULTS AND DISCUSSION

Total phenolics and flavonoïds contents

Phenolic compounds have received considerable attention, due to their antioxidant activities and freeradical scavenging abilities, which potentially have beneficial implications in human health (Imeh, Khokhar 2002; Lopez-Velez *et al.,* 2003). Proportions of total phenolics and flavonoïds measured according to the methods described previously showed that their contents were 57,85 ± 0,03 mg EAG/gDW and 15,95 ± 0,01 mg EQ/gDW respectively. These results are in agreement with recent data for the same species from Algeria (Rached *et al.,* 2010).

Chemical composition of the essential oil

The essential oil of *W. saharae* was extracted by hydrodistillation appearing as blue-green color viscous liquid with a percentage yield of 0,6% (w/w). The volatile components identified by GC–MS, their relative area percentages and their arithmetic retention indices are summarized in Table 1. In this study, fifty-two components representing 91,54% of the *W. saharae* leaves oil were identified. The dominant compounds of essential oil leaves belonging to sesquiterpenes were Nerolidol (25,95%) and β-Eudesmol (38,12%). Our results are in agreement with previously studies (Ramaut *et al*., 1985; Elamrani *et al.,* 2007; Znini *et al*., 2011) in which a values of Nerolidol and β-Eudesmol were (17,26%; 17,4%; 31,5%) and (42.25%; 52,7%; 45,6%) respectively. Among the other chemical components were Sabinene, 1,8-Cineole, α-Phellandrene, Linalool acetate, Cedren-13-ol, 8-, Naphthalene,1,2-dihydro-4-(4-methylphenyl)-, γ-Himachalene, α-Longipinene, β-Spathulenol, Ledene, Isoledene, α-Eudesmol, δ-Selinene, Cubenol, γ-Costol, and Dodecamethyl-1,11-dihydrohexasiloxane. Among identified compound, Nerolidol showed antileishmanial activity (Arruda *et al.,* 2005) and exhibits antineoplastic activity (Wattenberg, 1991). This compound is a sesquiterpene present in essential oils of several plants, approved by the U.S. Food and Drug Administration as a food flavoring agent. β-Eudesmol has multiple pharmacological effects. The anti-inflammatory effect of β-eudesmol was shown recently (Seo *et al.,* 2011).

Antioxidant activity

The DPPH radical is a free radical, which has been widely used as a tool to estimate free radical scavenging activity of antioxidants. Antioxidants, on interaction with DPPH, either transfer electrons or hydrogen atoms to DPPH, thus neutralizing the free radical character (Archana *et al.,* 2005). The DPPH free radical scavenging activity of leaves essential oil and ethanolic extract of *W. saharae* has been shown in Table 2. The IC50 values of essential oil and the ethanolic extract were compared with the standards ascorbic acid, BHT and α-tocopherol. Essential oil showed similar antioxidant activity (21,49 µg/ml) as compared to the standards and higher than that of ethanolic extract (182 µg/ml). This might be the result of the phenolic components existed in the essential oil of *W. saharae*. Edris (2007) reported that the antioxidant activity of volatile oils can be attributed to the presence of phenolic constituents and to the free radical scavenging activity of some volatile oils.

Table. 2: DPPH scavenging activity of ethanolic extract and essential of oil *W. saharea*.

Samples	%IP	IC50 (µg/ml)
Ethanolic Extrait	$80,93^{a}$ ± 0,014	182 (R^2 = 0,9805)
Essential oil	$79,50^{b}$ ± 0,005	21,49 (R^2 = 0,980)
BHT	$86,02^{c}$ ± 0,003	10,04 (R^2 = 0,997)
Vitamin C	$85,82^{c}$ ± 0,003	9,45 (R^2 = 0,989)
α-tocopherol	$82,23^{c}$ ± 0,002	18,82 (R^2 = 0,982)

Values represent mean ± SD for triplicate experiments.
IP = pourcentage d'inhibition, BHT = butyl hydroxyl toluen.
a = 1000 µg/ml, b = 500 µg/ml, c = 100 µg/ml.

Antibacterial activities

The antimicrobial activity of the essential oil and ethanlic extract of *W. saharae* were tested, in vitro, against four microorganisms, as seen in Table 3 and Table 4. Essential oil showed an important antibacterial activity against St. aureus ATCC 25923 and P. aeruginosa ATCC 27853, while the growth of E. coli ATCC 25922 and L. monocytogenes ATCC 19117 was not

Table. 3: Antibacterial activity of the essential oil of *W. saharae.*

Microorganisms	Concentrations (µg/ml) and zone inhibition (mm)						
	1000	0,5	0,25	0,125	0,063	0,031	0,016
	Disc diffusion test						
St. aureus ATCC 25923	28,5 ± 2,121	13 ± 2,82	11 ± 1,14	10,5 ± 0,70	10 ± 1,41	9,50 ± 0,70	-
E. coli ATCC 25922	9,50 ± 0,70	9,00 ± 0,00	8,50 ± 0,70	-	-	-	-
P. aeruginosa ATCC 27853	37,5 ± 3,53	22 ± 2,82	15 ± 0,00	13 ± 1,41	11,5 ± 0,70	11,5 ± 2,12	-
L. monocytogenes ATCC 19117	9,00 ± 1,41	-	-	-	-	-	-
	Macrobroth dilution test						
St. aureus ATCC 25923	+	+	+	-	-	-	-
E. coli ATCC 25922	-	-	-	-	-	-	-
P. aeruginosa ATCC 27853	+	+	+	+	-	-	-
L. monocytogenes ATCC 19117	-	-	-	-	-	-	-

Values are means ± SD of triplicate determinations. - : no detected antibacterial activity, + : detected antibacterial activity.

Table. 4: Antibacterial activity of the ethanol extract of *W. saharae* (Disc diffusion test).

Microorganisms	Concentrations (mg/ml) and zone inhibition (mm)				
	50	25	12,5	6,25	3,125
St. aureus ATCC 25923	12,5 ± 3,53	11,5 ± 3,53	9 ± 0,00	0,7 ± 0,00	-
E. coli ATCC 25922	11,5 ± 0,70	9,5 ± 0,70	9,5 ± 0,70		-
P. aeruginosa ATCC 27853	11,5 ± 0,70	10 ± 0,00	8,5 ± 0,70	-	-
L. monocytogenes ATCC 19117	9 ± 1,41	-	-	-	-

Values are means ± SD of triplicate determinations. - : no detected antibacterial activity.

inhibited. Ethanol extract showed moderate antimicrobial activity against all microorganisms tested at high concentration (50 mg/ml) (Table 4). The DMSO did not show antibacterial activity against the tested bacteria (negative control). The higher observed activity of essential oil could be attributed to the presence of sesquiterpenes, monoterpene hydrocarbons and oxygenated monoterpenes in the leaves essential oil. The essential oils containing terpenes are reported to possess antimicrobial activity (Dorman, Deans 2000) which is consistent in part with our present study. The mechanism of action of terpenes is not fully understood. The antibacterial effect of the oil could be explained through the disruption of bacteria membrane integrity. Indeed, previous findings revealed that tea tree oil damages the cell membrane structure of E. coli, S. aureus and Candida albicans (Cox *et al.*, 2000). It is also possible that the minor components might be involved in some type of synergism with the other active compounds (Marino *et al.*, 2001).

Table. 5: MICs values assays of ethanol extract and essential oil of *W. saharae*

Microorganisms	Ethanol extract (mg/ml)	Essential oil (µg/ml) tests	
		Disc diffusion	Macrobroth dilution
St. aureus ATCC 25923	6,25	0,031	0,25
E. coli ATCC 25922	12,5	0,25	-
P. aeruginosa ATCC 27853	12,5	0,031	0,125
L. monocytogenes ATCC 19117	> 25	> 0,5	-

- : no detected antibacterial activity.

The MICs values of the leaves essential oil against the tested bacterial strains were found lower as compared to the leaves ethanolic extract as reported in Table 5. St. aureus ATCC 25923 and P. aeruginosa ATCC 27853 were strongly inhibited by essential oil of *W. saharae*, since MICs values were lower (0,031 µg/ml and 0,25 µg/ml for disc diffusion method and macrodilution test respectively). That of E. coli ATCC 25922 was 0.25 µg/ml for the disc diffusion method. The MICs of ethanolic extract to inhibit St. aureus ATCC 25923 and P. aeruginosa ATCC 27853 were 6,25 mg/ml and 12,5 mg/ml respectively. The MICs of essential oil and ethanolic extraxct to inhibit L. monocytogenes ATCC 19117 was not determined, since there were no inhibitory activities. In our best knowledge, no results elucidating neither the antibacterial, antioxydante nor the toxicity of essential oil from *W. saharae* were published.

Toxicity toward *A. salina*

A. salina (brine shrimp), has gained popularity as a test organism that evaluates toxicity because of its ease of culture, short generation time, cosmopolitan distribution and the commercial availability of its dry cysts. In this study we have attempted to determine the LC50 of essential oil and ethanolic extract of *W. saharae* on Artemia metanauplii. The average LC50 values for essential oil and ethanolic extract of *W. saharae* were 1,56 µg/ml and 45,02 mg/ml, respectively (Table 6).

Table. 6: Lethal concentrations (LC50) and porcentage mortality values of essential oil and ethanolic extract of *W. saharae* toward A. salina after 24h.

Extract	LC50	% Mortality
Essential oil	1,56 µg/ml (R^2 = 0,921)	$86,66^a$ (± 1,15)
Extrait ethanolic	45,02 mg/ml (R^2 = 0,822)	10^b (± 1,00)
Temoin +	-	0 (± 0,00)
Temoin -	-	0 (± 0,00)

Values are means ± Standard Deviation (SD) of triplicate determinations.
Temoin + : DMSO, Temoin - : Seawater (35% NaCl).
a = 20 µg/ml, b=100 µg/ml.

According to Meyer *et al.*, (1982) essential oil is classified as toxic (LC50 <10 mg/L) whereas ethanolic extract is not toxic (LC50>100 mg/L). Control experiments were carried out with DMSO using the highest possible concentration in a test solution and exhibited no observable effects to *A. salina*. The predominance of the sesquiterpenes (Nerolidol and β-Eudesmol) found in our results in the identified components of the essential oil is in agreement with the studies of Hilmi *et al.*, (2003) in which they isolated a cytotoxic sesquiterpene lactones against a cancer cell line (KB cells) from the dichloromethane extract of the plant.

Toxicity against *A. salina* demonstrates a good correlation with the cytotoxic activity for some human solid tumors (Pimenta *et al.*, 2003). Therefore, antitumoral properties could be expected from essential oil of *W. saharae.*

CONCLUSION

The aim of this study was to evaluate the antioxidant by DPPH test and the antimicrobial activities of the essential oil of *W. saharae* against some pathogenic bacteria, evaluating minimal inhibitory concentrations. Our results showed that, this oil reveal a relative high antioxidant capacity and inhibit strongly the growth of St. aureus ATCC 25923 and P. aeruginosa ATCC 27853 at low concentrations. Thus leaves of *W. saharae* could be used as a source of natural polyphenols and antioxidants. Further investigation may be helpful in the use of the specific phenolic constituents of the essential oil as food preservatives, as aromatic foods imparts flavor and as natural antioxidants to reduce oxidative stress in human beings.

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