

## Antioxidant activity of *Haloxylon scoparium* alkaloid extracts from Figuig region (southeastern of Morocco)

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The aim of this paper is to study the chemical composition of alkaloids present in *Haloxylon scoparium* Pomel extracts and to evaluate their antioxidant capacity. The alkaloids were isolated from two parts of *Haloxylon scoparium* plant by two extraction protocols. The quantitative study made it possible to propose the best protocol for the extraction of the alkaloids. Moreover, GC-MS analysis of alkaloid extracts allowed us to determine their chemical composition. *Haloxylon scoparium* contains four types of alkaloids: tetraisoquinolines, phenylethylamines, tryptolines and tryptamines. The main compounds are the tetraisoquinolines type, the predominant product of which was N-methylsalsoline. These compounds present a great interest for the researchers due to their various pharmacological and biological activities. The antioxidant effect of the different plant extracts was studied by two methods: the ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picryl hydrazyl free radical (DPPH<sup>•</sup>) scavenging tests. The results show that extracts of root part are more active than those from aerial part; the acetone/water extract is the most powerful. The interesting results obtained in this study will be supplemented by other analyses and biological tests in order to better valorize this plant.

**Keywords:** *Haloxylon scoparium*. Alkaloids. Extract. GC-MS analysis. DPPH. FRAP.

### INTRODUCTION

Oxygen is an essential element for multicellular organisms because it can produce energy from the oxidation of organic matter. About 5% of the oxygen is unevenly reduced to reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. These reactive oxygen species are formed in tissue cells by various endogenous and exogenous causes such as metabolism, chemicals and ionizing radiation (Ramya *et al.*, 2015). They are highly reactive species, able to damage some biological molecules such as DNA, carbohydrates, proteins and lipids (Lobo *et al.*, 2010). ROS are valuable, but they can be harmful to the

body when they are produced excessively, or when the antioxidant defense is absent. This condition is referred to as “oxidative stress” (Bhattacharya, 2014). Therefore, nature has provided antidotes to these toxic molecules to combat oxidative stress thus help the human body to reduce oxidative damage. It is in this sense that the study of antioxidant activity of plant extracts has become important today, because we can find in plants powerful antioxidants in plants (Singh, Chidambara Murthy, Jayaprakasha, 2002). These antioxidants have been the focus of growing interest for their usefulness to health, with little or no side effects. On the contrary, synthetic antioxidants which have some toxic and dangerous effects, even if they are profitable and efficient (Sarmadi, Ismail, 2010). Among the substances extracted from natural sources, there are alkaloids that have promising pharmacological activities, especially for the treatment of neurodegenerative diseases, related to antioxidant, anxiolytic, anti-inflammatory and

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antidepressant properties (Chaves, Feitosa, da S Araújo, 2016; Tiong *et al.*, 2013; Jung *et al.*, 2009).

In this context, our study focuses on the valorization of *Haloxylon scoparium*, a plant from southeastern Morocco, widely used in traditional medicine by the local population. *Haloxylon scoparium* Pomel is a halophyte shrub belonging to the Chenopodiaceae family (Li *et al.*, 2010; El Shaer, 2010). In Morocco, it is commonly called “*Remth*”, and grows in the following regions: Moroccan Sahara, Saharan Atlas, Anti-Atlas, High Atlas, the trays of eastern Morocco and central Atlantic Morocco (Fennane *et al.*, 1999). This plant can be used as a remedy for various diseases. The extract of the aerial part has been used in traditional medicine to cure ocular disturbances (Ben Salah *et al.*, 2002). In Morocco, *Haloxylon scoparium* is useful for the treatment of diabetes, scarring, headaches and hypertension (Ziani *et al.*, 2015; Eddouks *et al.*, 2002). Various studies have indicated that this plant has a larvicidal, molluscicidal, antibacterial and antioxidant activities (Sathiyamoorthy *et al.*, 1997; Mezghani-Jarraya *et al.*, 2009; Jarraya *et al.*, 2008). However, very few researches have been done on the chemical composition of *Haloxylon scoparium*, and to our knowledge, no study has been conducted to value the roots of this plant.

Our work aims at the structurally studying of the alkaloid compounds contained in the aerial and root parts of *Haloxylon scoparium* and the evaluation of the antioxidant activity of its extracts.

## MATERIAL AND METHODS

### Plant material

The aerial and root parts of *Haloxylon scoparium* were harvested in Figuig region, in the southeastern of Morocco, during flowering period. This plant was identified in the Laboratory of Biodiversity and Natural Substances at Ibn Tofail University. The voucher specimen (voucher

number 315 (Fennane *et al.*, 1999)) has been deposited at the herbarium of Faculty of Sciences, Ibn Tofail University, Morocco. Both parts of the plant were separated, cleaned and then dried in an oven at 40 °C for 24 h. They were then ground, using an electric grinder to obtain a powder having 300 µm as the average particle size.

### Phytochemical test for alkaloids

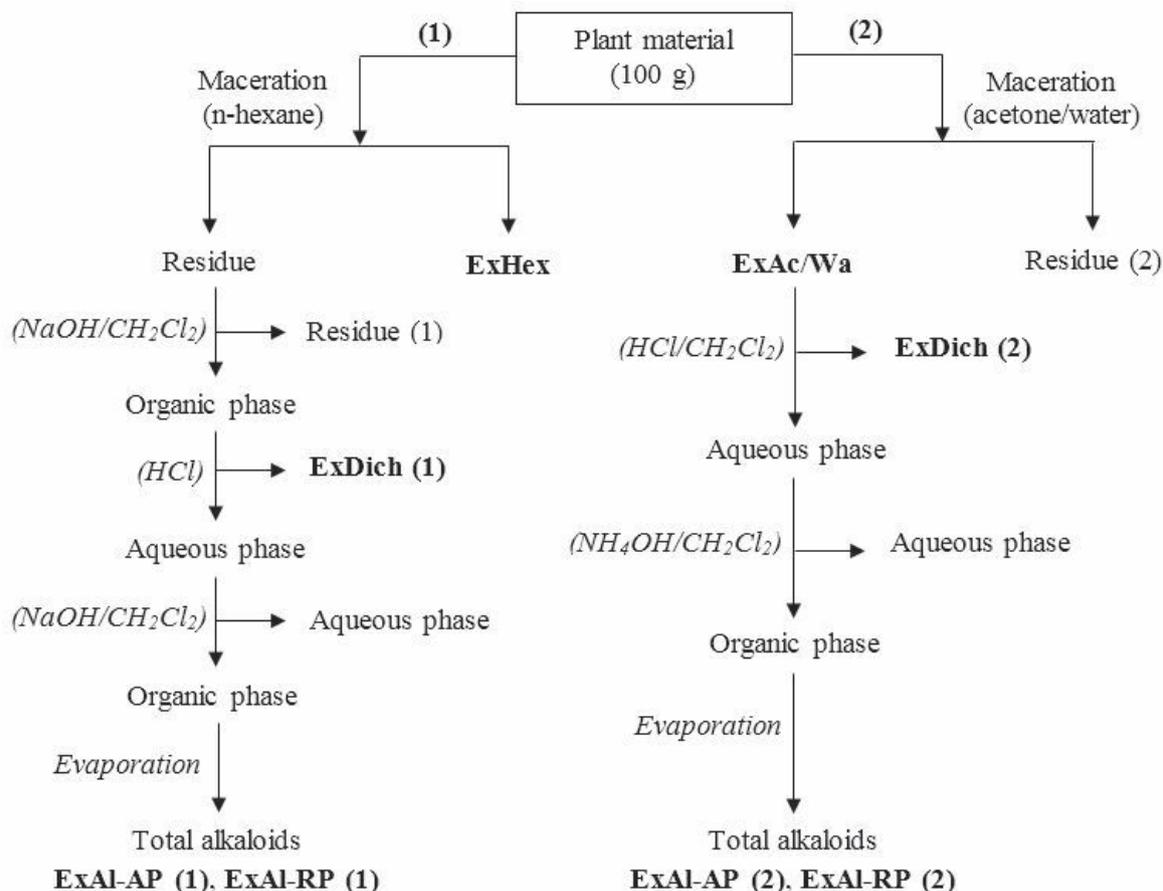
To demonstrate the presence of alkaloids in *Haloxylon scoparium*, the known protocol is to dissolve 1 g of powdered plant in 10 mL of 10% sulfuric acid, after agitation for 15 min, the mixture was filtered. The filtrate was poured in three tubes; two drops of Wagner's (Iodine in potassium iodide), Mayer's (Potassium mercuric iodide) and Dragendorff's (Potassium bismuth iodide) reagent were then added in each tube. The formation of brown, white, or orange precipitate, respectively, reveals the existence of alkaloids (Blond *et al.*, 2013).

These reactions are based on the ability of ions (Iodine, Mercury, and Bismuth) from the reagents to combine with nitrogen of alkaloid to form an ion pair that produces an insoluble precipitate, in slightly acid aqueous medium (Crauste, Vigor, Vercauteren, 2015).

### Alkaloids extraction

In plants, alkaloid compounds frequently occur as acid salts, but some also appear in combination with sugars, while others appear as amides or esters. They can also be quaternary salts or tertiary amine oxides (Tadeusz, 2007).

The alkaloids have the capacity to form ammonium salts in acidic medium which facilitates their extraction. In this study, two extraction protocols are used: the first is a delipidation of the plant followed by extraction with an apolar solvent in a basic medium, and the second is a maceration in a polar solvent followed by extraction in an acid medium (Figure 1).



**FIGURE 1** - Alkaloids extraction protocols of *Haloxylon scoparium*.

(1): First extraction protocol; (2): Second extraction protocol; ExHex: Hexane Extract; ExAc/Wa: Water Acetone Extract; ExDich: Dichloromethane Extract; ExAl-AP: Extract of Alkaloids from Aerial Part; ExAl-RP: Extract of Alkaloids from Root Part.

#### First extraction protocol

100 g of powder, aerial and root part, of *Haloxylon scoparium*, were macerated in 250 mL of hexane for 3 h. After filtration, the filtrate was evaporated. The residue was dried, basified with 60 mL of 10% (w/v) sodium hydroxide solution overnight, and then stirred with dichloromethane (350 mL) for 3 h. The mixture was filtered, and the solution obtained was extracted with 2N hydrochloric acid solution. The two phases were separated, the organic phase was evaporated and the aqueous phase was alkalinized with a basic solution (10 % NaOH) to pH 9-10, and then extracted many times with dichloromethane. The organic phase was dried by

anhydrous sodium sulphate ( $\text{Na}_2\text{SO}_4$ ), filtered and then evaporated to obtain the total alkaloids.

#### Second extraction protocol

The aerial and root parts of *Haloxylon scoparium* (100 g) were macerated in acetone/water mixture (70%, v/v) for 2 h. After filtration, evaporation and lyophilization, the solid extract obtained was solubilized in a 1N hydrochloric acid solution and then extracted with dichloromethane until the aqueous phase was exhausted. The aqueous phase, containing the alkaloids in the salt form, was then alkalinized to pH 11 using a concentrated ammonium hydroxide ( $\text{NH}_4\text{OH}$ ) solution. Finally, the total alkaloids were extracted with dichloromethane.

## Analysis of *Haloxylon scoparium* extracts

### TLC (Thin layer chromatography) analysis

To perform thin layer chromatography (TLC), aluminum silica plates were used. The extracts, to be analyzed, were deposited 1 cm above the base of plate. The plate was then eluted in a mixture of dichloromethane in methanol (98/2, v/v). The plate was revealed by two means: the first was to visualize the spots under ultraviolet light at 365 nm. The second was a plate spraying with Dragendorff's reagent, specific for detecting the presence of alkaloids.

### Derivatization protocol of extracts

The alkaloids extracts are generally composed of basic compounds, having functions such as amines and alcohols, containing one or more active hydrogen atoms; therefore, studying them by GC-MS requires derivatization reaction. In this work, the chosen reaction was acetylation. This method allows the formation of acetylated derivatives to be better analyzed by gas chromatography. In this case, a mass ion  $m/z$  43 shows up in the mass spectrum corresponding to the acetyl group of the acetylated compound (Zanetta *et al.*, 2001).

The reaction was performed following the protocol described by (Jerkovic, Mastelic, 2004) with some modifications. 5 mg of the sample was solubilized in 1 mL of acetic anhydride, 4 drops of pyridine were added, which was used as catalyst, and the mixture was heated for 20 minutes in a water bath at 50 °C. The mixture was left to cool down during one night, and then hydrolyzed with 6 mL of cold water with magnetic stirring for 2 hours in an ice water bath. The products were extracted with chloroform, the organic phase was neutralized with a saturated solution of sodium hydrogenocarbonate ( $\text{NaHCO}_3$ ), dried by anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) and evaporated.

### GC-MS (Gas Chromatography - Mass Spectrometry) analysis

The analysis of alkaloids was performed using gas chromatography coupled with mass spectrometry, at University Center for Analysis, Expertise, Technology

Transfer and Incubation at Ibn Tofail University Ibn Tofail, Kenitra, using the apparatus Bruker consisting of 456-GC type chromatogram coupled to EVOQ TQ type mass spectrometer operating in electronic impact mode. The capillary column used was a Thermo TR-35 MS (30m  $\times$  0.25mm  $\times$  0.25 $\mu\text{m}$ ). The temperature of column was initially maintained at 60 °C for 10 min, with a rise of 10 °C/min to 80 °C (2 min), then an increase of 5 °C/min to 300 °C (5 min). The injector temperature was 280 °C, and the ion source temperature was 250°C. The flow rate of carrier gas (Helium) was 1 mL/min, and the injection volume was 1  $\mu\text{L}$ . Mass spectra were obtained at 70 eV.

The identification of alkaloid structures was confirmed by comparing the obtained mass spectra with those of the pure compounds by reference to the library of mass spectra of the apparatus (NIST 2014) and based on the literature (Jarraya *et al.*, 2008; Bringmann *et al.*, 2000; Coutts, Locock, Slywka, 1970; Gupta *et al.*, 2015).

## Antioxidant activity

### DPPH free radical scavenging test

The antioxidant activity, of the specified *Haloxylon scoparium* extracts, was determined using the stable 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) scavenging method, as described by (Olugbami, Gbadegesin, Odunola, 2015), with some modifications. Briefly, a solution of DPPH at 76  $\mu\text{M}$  (0.03 mg/mL) was prepared in ethanol by adding 2 mL of this solution were added to 0.1 mL of extracts at different concentrations (from 0 to 3 mg/ml). A blank, containing the DPPH solution, and the solvent used for the solubilization of extracts, was prepared in parallel. The mixture was stirred and then incubated in the dark, at room temperature for 30 minutes. Absorbance was measured at 517 nm using a spectrophotometer (UV-2005, Selecta), and ascorbic acid was used as positive control.

The percentage inhibition of DPPH was calculated by applying this formula:

$$(\%) \text{ Inhibition} = [(Ab - As) / Ab] \times 100$$

Ab: Absorbance of blank, As: Absorbance of sample.

The IC<sub>50</sub> value, which represents the sample concentration needed to inhibit 50% of free radical DPPH, was calculated using the probit analysis.

#### *Ferric reducing antioxidant power (FRAP)*

The ferric ion reducing antioxidant power, in the extracts, was evaluated according to the method described by (Oyaizu, 1986). 1mL of extracts at different concentrations (0 - 1 mg/mL) was mixed with 2.5 mL of phosphate buffer solution (0.2 M at pH 6.6) and 2.5 mL of potassium ferricyanide solution ( $K_3Fe(CN)_6$ ) at 1% w/v. The mixture was incubated at 50 °C for 20 min. Then 2.5 mL of 10% w/v trichloroacetic acid was added to stop the reaction and the tubes are centrifuged at 3000 rpm for 10 min, and then 2.5 mL of supernatant was combined with 2.5 mL of distilled water and 0.5 mL of 0.1% w/v  $FeCl_3$ . The absorbance was measured at 700 nm against a blank prepared in the same way, containing all the reagents and the solvent used for the solubilization of extracts. Ascorbic acid was used as a positive control.

To express the results obtained, sample concentration, giving 0.5 of the absorbance (IC<sub>50</sub>), was calculated from the graph representing the absorbance as a function of extract concentration (Estevinho *et al.*, 2008).

## RESULTS AND DISCUSSION

### Phytochemical tests

The results of phytochemical tests, carried out on the two parts of *Haloxylon scoparium*, clearly indicate

the presence of the alkaloid compounds, characterized by a positive response to the revelation tests with the three reagents (Wagner, Mayer and Dragendorff), for the two parts of *Haloxylon scoparium*.

### Quantitative results of extractions

The quantitative study of the two extraction protocols was summarized in Table I. From these results, it seems clear that the yields are better with the second extraction protocol; the aerial part provides the highest quantities.

From 100 g of each plant part, a total of 4.23 g and 7.14 g of alkaloids extract were obtained respectively for the first and second extraction protocol. These variations of yields in the second protocol can be assigned to the use of polar solvent (acetone-water), which is able to extract more polar compounds than dichloromethane solvent in the first protocol.

The residues of the two extraction protocols were obtained in very large quantities. They have been examined by phytochemical tests to ensure the absence of alkaloid compounds. The results obtained (Table I) have shown that the first extraction protocol (performed in a single step) was not complete and other extraction steps were necessary to isolate all the alkaloids. Taking into account all the results obtained, the second protocol of extraction seems better for alkaloids extraction.

**TABLE I** - Quantitative results and phytochemical test of *Haloxylon scoparium* extracts obtained by two extraction protocols of both plant parts

Extraction protocol	Extract name	Yield (% w/w)		Result of Phytochemical test
		Root part	Aerial part	
(1)	ExHex	0.18	1.13	-
	ExDich (1)	0.25	1.29	-
	ExAl (1)	0.16	4.07	+
	Residue (1)	96.25	87	+
(2)	ExAc/Wa	10.99	23.54	+
	ExDich (2)	0.92	0.78	-
	ExAl (2)	0.80	6.34	+
	Residue (2)	86.15	75.85	-

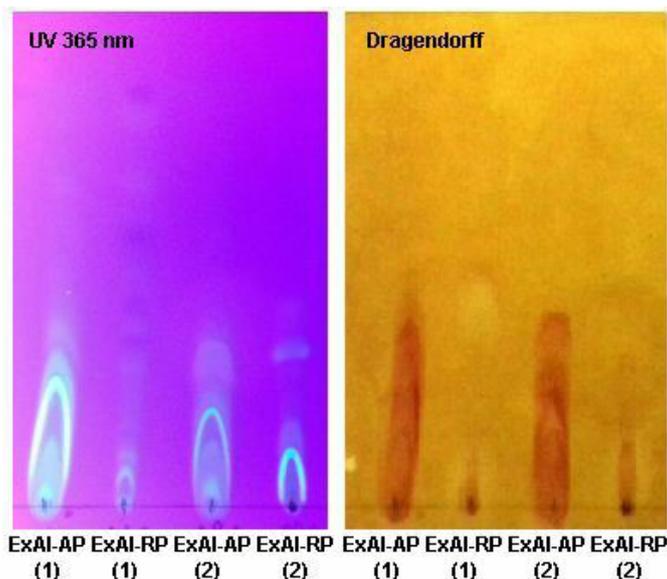
(+): Presence of Alkaloids; (-): Absence of Alkaloids; **(1)**: First extraction protocol, **(2)**: Second extraction protocol; **ExHex**: Hexane Extract; **ExDich**: Dichloromethane Extract; **ExAl**: Alkaloids Extract; **ExAc/Wa**: Acetone Water Extract.

## Qualitative analysis results

### TLC analysis results

TLC analysis of the alkaloid extracts (ExAl-AP, ExAl-RP), obtained by both extraction protocols for

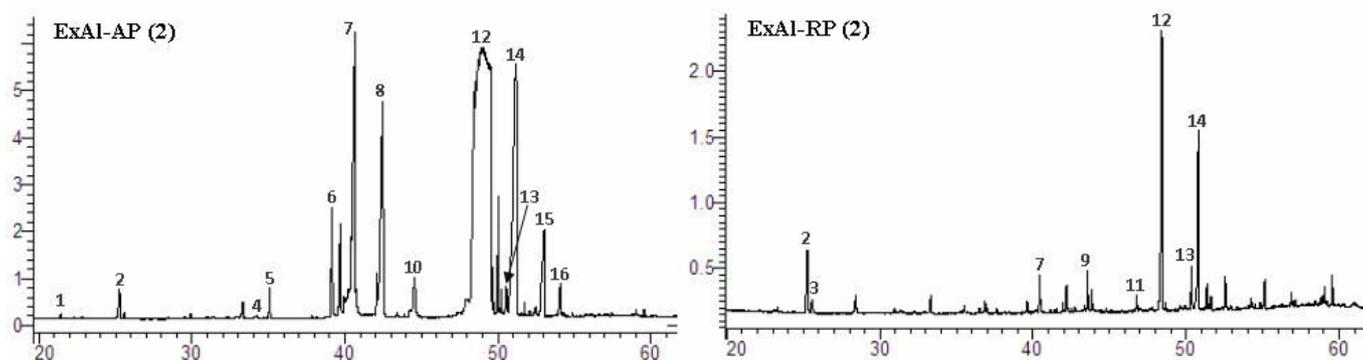
the two parts of *Haloxylon scoparium* (Figure 2), have shown the presence of numerous colored spots which are visualized under UV at 365 nm, especially in blue and fluorescent blue. After revelation of these spots by the Dragendorff's reagent, an orange color appeared which confirms the presence of the alkaloids in these extracts.

**FIGURE 2** - Confirmation of the presence of the alkaloids in the various extracts obtained by thin layer chromatography (TLC) analysis.

(1): First extraction protocol; (2): Second extraction protocol; ExAl-AP: Extract of Alkaloids from Aerial Part; ExAl-RP: Extract of Alkaloids from Root Part; Dragendorff: Reagent used for alkaloid revelation, UV 365 nm: Ultraviolet light at a wavelength of 365 nm.

## GC-MS analysis results

GC-MS analysis results of alkaloid extracts obtained by the second extraction protocol were shown in Figure 3 and Table II.



**FIGURE 3** - Chromatograms of the acetylated alkaloids extracts, obtained by the second extraction protocol in the aerial and root parts of *Haloxylon scoparium*.

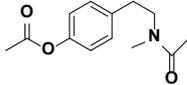
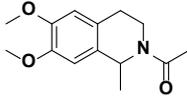
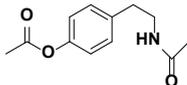
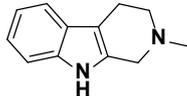
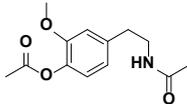
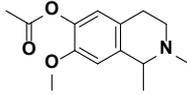
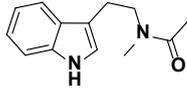
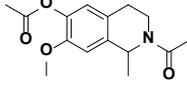
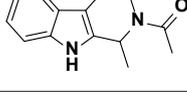
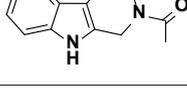
(2): Second extraction protocol; ExAl-AP: Extract of alkaloids from Aerial part; ExAl-RP: Extract of alkaloids from Root part.

**TABLE II** - Chemical composition of the acetylated alkaloids extracts (ExAl (2)) obtained in the aerial part (AP) and the root part (RP) of *Haloxylon scoparium* by the second extraction protocol

N	Compound name	RT (min)	m/z by MS (%)	Chemical structure	Plant part	Peak area %
1	terpinen-4-ol	21.39	71(100), 43(65.2), 93(34.7) 55(33.4), 111(19.9), 154(2)		AP	0.07
2	triacetine	25.25	43(100), 103(4.7), 86(1.3) 116(0.3), 73(0.7), 145(0.4)		RP AP	7.98 0.59
3	ethylidenediacetate	25.57 25.59	43(100), 87(4.2), 56(2.3), 117(2.2), 74(1.6), 103(1.2)		RP AP	1.46 0.08
4	N-phenethylacetamide	34.24	30(100), 104(69.9), 43(59.6), 91(26.7), 65(25.2), 51(13.1), 77(10.4), 163(3.6)		AP	0.04
5	N-methyl-N-phenethylacetamide	35.08	44(100), 43(19.4), 86(17.3), 65(6.3), 104(3.4), 177(0.7)		AP	0.39
6	carnegine	39.16	206(100), 91(28.7), 190(28.2), 103(25.6), 77(23.59), 162(17.3), 207(12), 221(0.6)		AP	1.85

(continues on the next page...)

**TABLE II** - Chemical composition of the acetylated alkaloids extracts (ExAl (2)) obtained in the aerial part (AP) and the root part (RP) of *Haloxylon scoparium* by the second extraction protocol

N	Compound name	RT (min)	m/z by MS (%)	Chemical structure	Plant part	Peak area %
7	4-(2-(N-methylacetamido)ethyl)phenylacetate	40.47 40.64	44(100), 86(43.6), 43(40.7), 107(16.5), 77(15.4), 129(13.7), 192(12.9), 116(9.4)		RP AP	2.91 7.76
8	N-acetylsalsolidine	42.49	192(100), 234(70.1), 177(40.1), 43(17.7), 149(17.2), 164(14.3), 91(13.3), 77(10.1)		AP	7.19
9	N,O-diacetyltyramine	43.57	43(100), 120(83.9), 30(67.5), 107(37.8), 77(18.9), 72(10.2), 91(9.8), 51(8), 162(7.8)		RP	3.13
10	2-methyltryptoline	44.55	143(100), 42(27.7), 115(21.8), 144(14.2), 77(12.1), 63(10.1), 51(9.1), 186(7.2), 128(5.6)		AP	1.44
11	acetamide, N-[2-[4-(acetyloxy)-3-methoxyphenyl]ethyl]-	46.76	43(100), 150(55.2), 30(39.4), 137(20.7), 77(11.3), 65(10.3), 51(9), 94(8.4), 122(5.9)		RP	1.43
12	O-acetyl-N-methylsalsoline	48.42 49.09	192(100), 43(51.9), 234(32.75), 176(16.5), 91(27.7), 77(24.5), 103(14.7), 131(11.9), 118(11.1), 249(8.4)		RP AP	30.60 56.10
13	N-methyl-N-acetyltryptamine	50.25 50.36	44(100), 130(52), 143(42.5), 43(37.1), 77(22.7), 103(10), 51(7.6), 144(5.7), 63(4.5), 216(0.4)		AP RP	0.17 3.36
14	N,O-diacetylsalsoline	50.79 51.25	178(100), 220(60.4), 262(38.4), 43(29.8), 163(20.6), 148(8.5), 91(8.4), 131(7.4), 277(3.8)		RP AP	15.60 11.30
15	1H-pyrido[3,4-b]indole, 2,3,4,9-tetrahydro-1-methyl-2-acetyl-	53.03	171(100), 43(70.5), 115(40.2), 130(39.2), 156(38.1), 228(25.1), 77(24.8), 213(21.4), 185(19.2)		AP	2.73
16	1-(1,3,4,9-tetrahydro-2H-pyrido[3,4-b]indol-2-yl)ethan-1-one	54.09	143(100), 43(64.1), 115(39), 144(27.6), 77(18.7), 154(15.9), 89(15.3), 171(15.6), 214(14.6), 63(14.2)		AP	0.54

N: Number of the peak in the figure 3; RT: Retention Time; AP: Aerial Part, RP: Root Part; m/z by MS (%): Characteristic peaks obtained by Mass Spectrometry analysis (relative abundance of peaks in %).

In total, 90.2% and 66.5% of directly analyzable compounds were identified in the aerial and root parts, of which approximately 89.4% and 57% are alkaloids.

Thirteen identified alkaloid compounds were divided into four main groups, namely tetrahydroisoquinolines (**6**, **8**, **12**, **14**), phenylethylamines (**4**, **5**, **7**, **9**, **11**),

tryptolines (**10**, **15**, **16**) and tryptamines (**13**). Among the tetrahydroisoquinoline compounds, O-Acetyl-N-Methylsalsoline **12** is the most abundant alkaloid compound (50.6% and 30.6% of the compounds identified in ExAI (2) from the aerial and root parts of plant). Three other tetrahydroisoquinolines have also been identified, namely acetylated derivatives of Carnegine **6**, Salsolidine **8** and Salsoline **14**. This group of compounds is naturally the most numerous among the alkaloids; they also display the widest range of structural diversity. Many of them have biological activities that are attached to the isoquinoline nucleus (Bracca, Kaufman, 2004).

The phenylethylamines present in *Haloxylon scoparium* are 2-Phenylethanamine **4**, N-Methylphenethylamine **5**, N-Methyltyramine **7**, Tyramine **9** and 3-Methoxytyramine **11**. These compounds are identified as acetylated derivatives. The phenylethylamines cover a wide range of different activities: antihistaminic, adrenergic, anorexic, etc. (Seijas *et al.*, 2001). They are also known by their functions as neurotransmitters (Irsfeld *et al.*, 2013). These compounds are precursors of isoquinolines (Tadeusz, 2007).

The tryptolines are natural alkaloids with a broad range of pharmacological and biological activity (Buckholtz, 1980). They are of great interest because of their presence in food consumption (Tomas Herraiz, 2003). They are neurotransmitters or neuromodulators and can be formed endogenously in the brain (Buckholtz, 1980; Herraiz, 1997). The tryptolines identified in the extracts analyzed are 2-Methyltryptoline **10** and the derivatives of Tetrahydroharman **15** and Tryptoline **16**. These compounds form readily from tryptamines by the Pictet-Spengler reaction (Cao *et al.*, 2007). Only one tryptamine has been identified, in *Haloxylon scoparium*, it is the acetyl compound of N-Methyltryptamine **13**.

Tryptamines are known by their hallucinogenic psychotropic properties. They play a fundamental role

in human life such as serotonin, one of the most important signaling hormones in the body; it is involved in the regulation and modulation of multiple processes of the central nervous system, such as temperature regulation, sleep, memory, cognition and behavior (Nichols DE, Nichols CD, 2008).

Other types of chemical compounds have been identified, namely acetylated alcohols (**1**, **2**, **3**) which are also extractable in an acid medium.

In previous studies realized on *Haloxylon scoparium*, two alkaloids were isolated (carnegine and N-methylisosalsoline) from the crude extract of this plant (Jarraya *et al.*, 2008; Bouaziz *et al.*, 2016; El-Shazly *et al.*, 2003). 2-methyl-1,2,3,4-tetrahydrocarboline and salsolidine have also been isolated from the hydroethanolic extract of this same plant (El-Shazly *et al.*, 2003). Other studies have identified isosalsoline, dehydrosalsolidine, isosalsolidine (tetrahydroisoquinolines), N-methylcorydaldine (isoquinolone), tryptamine and N-methyltryptamine (tryptamines) as minor alkaloids (Benkrief *et al.*, 1990).

### Antioxidant activity

In this study, the antioxidant activity of *Haloxylon Scoparium* extracts was evaluated by two different biochemical tests: FRAP (Ferric reducing antioxidant power) and DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging test. FRAP test measures the ability of compounds to act as an electron donor while DPPH measures their capacity to form a stable radical by giving hydrogen (Chan, Lim, Chew, 2007).

Figure 4 shows the straight that express the variation in percent inhibition of DPPH in a known concentration range, for the different plant extracts. IC<sub>50</sub> values calculated by probit analysis were given in Table III. Ascorbic acid, taken as reference molecule, has the most important activity; IC<sub>50</sub> = 83.75 µg/mL.

**TABLE III** - Results of the antioxidant activity of *Haloxylon scoparium* extracts, containing the alkaloids, by both tests (DPPH and FRAP)

Extract	DPPH [IC50]*		FRAP [IC50]*	
	Aerial part	Root part	Aerial part	Root part
ExAl (1)	6102.09 ± 36.34	1080.92 ± 60.94	961 ± 20	551 ± 12
ExAl (2)	4044.88 ± 586	582.28 ± 26.93	1112 ± 229	460 ± 3
ExAc/Wa	550.82 ± 33.66	222.41 ± 63.69	681 ± 189	244 ± 11
Ascorbic acid	83.75 ± 6.29		100 ± 6.00	

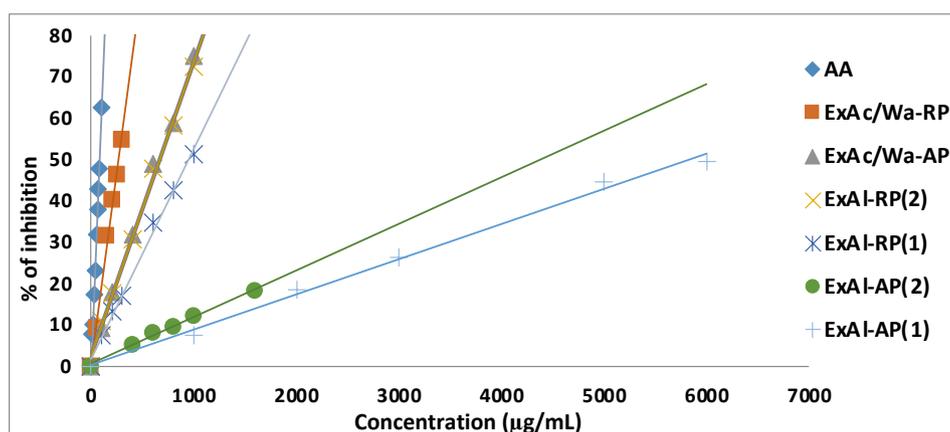
\*: Value in  $\mu\text{g/mL}$ ; (1): First extraction protocol; (2): Second extraction protocol; **ExAl**: Alkaloids Extract; **ExAc/Wa**: Acetone Water Extract; **IC50**: Concentration needed to inhibit 50% of free radical DPPH; **DPPH**: 1,1-diphenyl-2-picryl hydrazyl free radical; **FRAP**: ferric reducing antioxidant power.

Results obtained show that extracts (ExAc/Wa) have the highest reducing power of all plant extracts. We also note the importance of the reducing power of ExAc/Wa from the root part. This result has already been observed in the antioxidant power study of other plants in laboratory. Water/Acetone extracts (starting extracts) contain the various compounds likely to have significant antioxidant activity. A synergistic phenomenon between the different compounds of the extract can also explain these results.

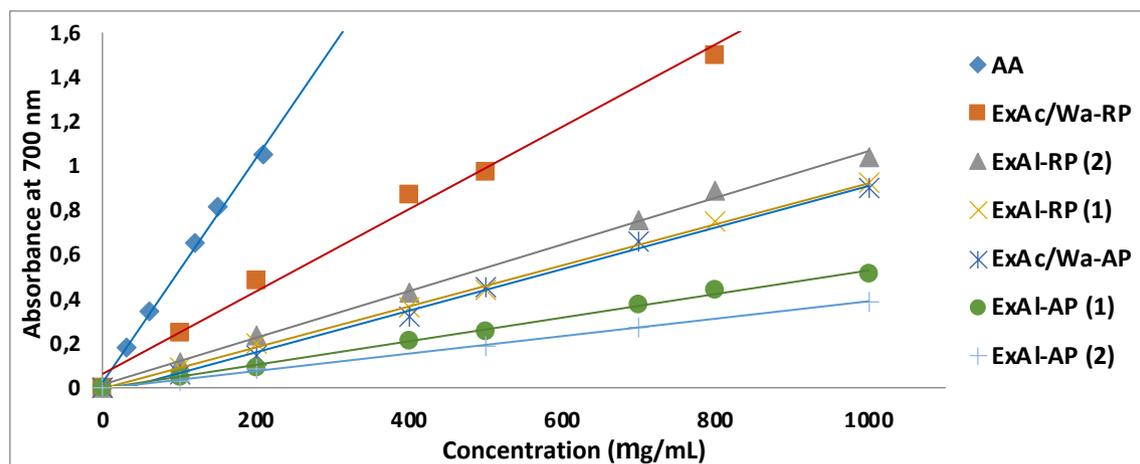
Comparing the IC50 values obtained for alkaloid extracts (ExAl), we found out that the reducing power

of the root part of extracts was more powerful than that of the aerial part of extracts (582.28 and 1080.92  $\mu\text{g/mL}$ ) against (4044.88 and 6102.09  $\mu\text{g/mL}$ ), respectively for both extraction protocols. This result, which was repeated for further extracts, can be attributed to the difference in chemical composition of the two parts of plant.

For FRAP test, Figure 5 shows the variation of the absorbance as a function of concentration for the various extracts of *Haloxylon scoparium* obtained by two extraction protocols. The presence of reducing compounds in the extracts causes the reduction of  $\text{Fe}^{3+}$  present in  $\text{K}_3\text{Fe}(\text{CN})_6$  complex to the ferrous form ( $\text{Fe}^{2+}$ ).

**FIGURE 4** - Straight, expressing the variation of the percentage of inhibition as a function of the concentrations of *Haloxylon Scoparium* extracts, obtained by the DPPH test.

AA: Ascorbic Acid; **ExAc/Wa-RP**: Water Acetone Extract from Root Part; **ExAc/Wa-AP**: Water Acetone Extract from Aerial Part; **ExAl-RP**: Extract of Alkaloids from Root Part; **ExAl-AP**: Extract of Alkaloids from Aerial Part; (1): First extraction protocol; (2): Second extraction protocol.



**FIGURE 5** - Straight, expressing the variation of the absorbance as a function of the concentrations of *Haloxylon Scoparium* extracts, obtained by the FRAP test.

AA: Ascorbic Acid; **ExAc/Wa-RP**: Water Acetone Extract from Root Part; **ExAc/Wa-AP**: Water Acetone Extract from Aerial Part; **ExAl-RP**: Extract of Alkaloids from Root Part; **ExAl-AP**: Extract of Alkaloids from Aerial Part; **(1)**: First extraction protocol; **(2)**: Second extraction protocol.

The IC<sub>50</sub> values obtained by the FRAP test for the various extracts studied (Table III) show that the latter have various reducing powers. From FRAP test, the antioxidant activity of the various extracts can be classified in descending order as follows: (ExAc/Wa-RP > ExAl-RP (2) > ExAl-RP (1) > ExAc/Wa-AP > ExAl-AP (1) > ExAl-AP (2)). Although this order is different from the one obtained in the case of the DPPH test, the highest activity of ExAc/Wa-RP extract and the lowest activity of ExAl-AP extract can nevertheless be noticed in the results of the two tests. Comparing the antioxidant activity of the same extracts from the two different parts of the plant, it can be emphasized, as in the case of the DPPH test, that the extracts of the root part are more active than those of the aerial part.

The remarkable antioxidant activity of the root extracts of *Haloxylon scoparium* is probably due to the chemical composition of these extracts. The presence of compounds having phenol groups in their structures, such as N-methylsalsoline, salsoline and other compounds present only in these extracts, such as tyramine and 3-methoxytyramine, may explain this result. Previous studies on alkaloids have shown that the presence of N-methylisosalsolein contributes to the increase of antioxidant activity (Bouaziz *et al.*, 2016). The same

remark has been observed for tryptolines because of the presence of an indole ring which can give the indolyl cation or a neutral radical by single electron transfer while acting as radical scavengers (Herraiz, Galisteo, 2003).

Other polar products unidentified by GC-MS coupling can also explain this results, an analytical study of extracts by HPLC and LC-MS can answer this hypothesis.

## CONCLUSION

The alkaloids, which are present in aerial and root parts of *Haloxylon scoparium*, were isolated by two extraction protocols. These compounds exist in a large amount in the plant aerial part; their chemical composition has been determined by coupling GC-MS. Thirteen alkaloids have been identified. The main ones of which were N-methylsalsoline (broadly majority), salsolin, carnegine, salsolidine and tyramine. The antioxidant activity of the various extracts was evaluated by two protocols: 1,1-diphenyl-2-picryl hydrazyl free radical (DPPH) scavenging test and the ferric ion reducing antioxidant power (FRAP) test. The root extracts of *Haloxylon scoparium* showed a significant effect compared to those from the aerial part.

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