Algerian Journal of Environmental Science and Technology
June edition. Vol.8. N°2. (2022)
ISSN : 2437-1114
www.aljest.org

Extraction and valorization of active principles of *Saccocalyx satureioides* collected in two wilayas of Algeria.

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ARTICLE INFO

Article History :

Received ***: 18/05/2021***
Accepted ***: 16/01/2022***

***Key words*:**
Saccocalyx satureioides; essential oil; antibacterial activity; antifungal Djelfa M'sila; Zaafrane; Ain Chouhada.

ABSTRACT/RESUME

Abstract: *The study of Saccocalyx satureioïdes belonging to the Lamiaceae family and collected from two regions of Algeria, namely: Djelfa and M'sila, allowed to characterize the different families of chemical compounds contained in its aerial part, The analysis of Saccocalyx saiureioïdes essential oils of the two regions by gas chromatography coupled with mass spectrometry (GC/MS), allowed to identify the following major constituents (borneol 13. 24%, α-Terpineol 27.42% and thymol 34.87%) for the Zaafrane site and (borneol 11.9%, α-Terpineol 14.9% and thymol 41.09%) for the Ain chouhada site, in Saccocalyx satureioides.*

I. Introduction

The growing societal demand for natural, environmentally friendly products has led to a renewed scientific interest in essential oils as a natural, effective, economical and ecological potential.

Essential oils are mixtures of naturally occurring volatile compounds from plants and herbs.

Secondary metabolism [1], with antimicrobial, antioxidant, insecticidal, anti-inflammatory and anti-parasitic properties [2]. Essential oils are generally recognised as safe (GRAS) by the US Food and Drug Administration, so they are used in the pharmaceutical, food, agronomic and cosmetic industries [1].

Algeria is one of the African countries with a very rich biodiversity with a variety of plants that can be used for therapeutic purposes. This richness must be preserved in order to benefit from its virtues, and on the other hand to avoid the side effects of chemical drugs.

These plants have several chemical components that can be obtained by extraction of essential oil, the latter varying according to the environment of the species.

Among this wealth, we are interested in an aromatic plant, Saccocalyx satureioides, harvested in two wilayas, Djelfa and M'sila, according to several factors, to see the variation of its chemotype with the change of the latter, as well as its efficiency to determine the best essential oil. The aim is to extract the essential oils by hydrodistillation and microwave, to determine their chemical composition by GC/MS, to test their antibacterial, antifungal and antioxidant power as well as their concentrations in polyphenol, to see if the essential oil of this plant varies according to the factors tested, and to be able to determine the most active essential oil.

Saccocalyx satureioides is an Algerian endemic plant growing in the pre-desert zone; it is an aromatic shrub, locally called 'zaatar' with a thyme-like smell [3].

In folk medicine, the aerial part is commonly used as a decoction for the treatment of gastric disorders and spasms [3]. The antibacterial activity of essential oils or isolated components has been demonstrated on numerous occasions [4]. Two recent studies reported the chemical composition of Algerian S. satureioides oils obtained by hydrodistillation from plants harvested in the North and North-East of Algeria [5].

II. Material and methods

II.1. Methodology

To meet the objectives of my study, I used the following methods,
After harvesting Saccocalyx satureioides from the three sites, Zaafrane and Ain chouhada (Djelfa) and Benzouh (M'sila), in mid-April, the leaves, stems and roots were dried in the dark at room temperature for 8 days, and another quantity for one year.

II.2. plant material

Aerial parts consisting of stems and leaves of S. satureioides were collected in mid-April 2018 near two different regions: Djelfa and M'sila Algeria.
The stems and leaves were dried in the dark and at room temperature for 8 days. This plant has several chemical components that can be obtained by extraction of essential oil, which varies to the environment of the species.

II.3. Extraction and isolation of the essential oil

Hydrodistillation apparatus and procedure:

II-3.1.Clevenger

The essential oil of Saccocalyx satureioides was extracted by hydrodistillation using a Clevenger-type apparatus [6].
It is an extraction method whose role is to entrain the volatile compounds of natural products with the water vapor. It allows extracting the maximum of the compounds contained in the plant.

Figure 1. *Hydrodistillation by Clevenger distillation apparatus.*

II.3.2. Microwave assisted hydrodistillation (MAHD)

Is a process developed by Stashenko et al [7].
This process is entirely based on the principle of conventional hydrodistillation which consists in placing a part of the hydrodistillation apparatus in the microwave oven.

The plant material is thus placed in the presence of a sufficient amount of water in a reactor located in the microwave oven chamber.

Figure 2. *Hydrodistillation by microwave Clevenger distillation*

The refrigeration system and the part for the recovery of the essences are located outside the oven. The Clevenger set-up is placed in the microwave oven set at 500 watts for 10 minutes, so that the flask is inside the oven and the rest of the set-up is outside,
Put 50 g of plant material in a 2-litre Pyrex glass flask; add a volume of distilled water corresponding to 2/3 of the capacity of the flask. Then adapt the steam condensation apparatus (refrigerator) to the flask, the temperature of which does not exceed 23°C. We supply it with water and heat it with a bottle warmer, and we close the flask with a thermometer to check the temperature. After 15 to 20 minutes, when the temperature reaches 90°C, the water starts to boil, which causes the water vapor and the essential oil to liquefy in the cooling column. The result is a liquid composed of two phases: the water phase and the oil phase, which is the distillate. At the end of the extraction, we recover our essential oil in a brown glass at bottle, well closed and kept a temperature of 0 to 4°C.
The duration of the extraction is 2 to 4 hours maximum, and the operation is repeated several times (3 times maximum).
Do not forget to note its weight with a precision scale to be able to calculate the yield which is given by the following formula:

$$Rd = (M')/M \times 100 \qquad [8]$$

Rd: yield of essential oil expressed as a percentage
M ': mass of the essential oil obtained in grams (g).
M: mass of dry vegetable matter used in gram (g).

II-4.Gas chromatography/mass spectrometry

Gas chromatography is a separation analysis method that is applied to compounds that are gaseous or can be vaporised by heating without decomposition [9].

II.4. 1. Procedure

Gas-chromatography–mass spectrometry (GC-MS) was carried out on the same gas-chromatograph connected to a Hewlett-Packard mass spectrometer model 5971A.GC-FID was carried out with the following analytical conditions: ZB-5 capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness); helium as carrier gas; injection in split mode (1:50); injector and detector temperatures 250 and 280 °C, respectively.

The oven temperature was programmed from 40 to 300 °C at 2 °C/min. GC-MS was carried out in electron impact mode at 70 eV; electron multiplier 1700 V; ion source temperature, 180 °C; mass spectra data were acquired in the scan mode in m/z range 40–400.

II.4.2. Identification of components

Retention indices (RIS) were calculated by comparing the retention times of elution peaks with a series of linearly interpolated n-alkanes (C5-C28) (using a formula of Van den Dool and Kratz (1963)) on polar and non-polar columns. The identification of individual compounds was based on the comparison of their retention indices and mass spectra with those of authentic compounds or literature data stored in the electronic spectrometric library (NIST) and with those reported in the literature [10].

II.5. Aromatogram

II.5. 1. Microbial strains

The antimicrobial activities of Saccocalyx satureioides essential oils were tested against Gram-positive and Gram-negative bacteria as Bacillus subtilus ATCC 26633, Staphylococcus aureus ATCC 25923, Listeria monocytogenes ATCC 19111, Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 25922, Klebsiella pneumonea ATCC 13833, Proteus mirabilis ATCC 14153, Pseudomonas aeruginosa ATCC 27853, Salmonella typhimarium ATCC 19430obtained from the Ameri- can Type Culture Collection (Rockville, MD, USA). They were also tested against two strains of Candida albicans: one (Ca.1) isolated by the service of dermatology (CHU, Tlemcen) and the other (Ca.444), obtained from Pasteur Institute of Algeria (IPA). All the strains were grown on Mueller-Hinton agar (MHA) for the bacteria and Saboureaud Dextrose Agar (SDA) with chloramphenicol for yeasts.

II.5. 2. Procedure

The antimicrobial power of the essential oil is obtained by measuring the diameters of the inhibition zones in mm.

Note that for Ain chouhada we did not test the antimicrobial activity of the two bacteria P. aeruginosa (Gram-), B. subtilis (Gram+), because we did not have enough plant matterIt is an agar diffusion method that aims to qualitatively evaluate the vitro" antimicrobial activity of the essential oil of saccocalyx saturioides on the different bacterial and fungal strains tested by the agar diffusion method (aromatogram). That of direct contact, which has two methods: the well method, and the disk method. For this study we used the agar disk diffusion method to test the sensitivity of different strains. [11]

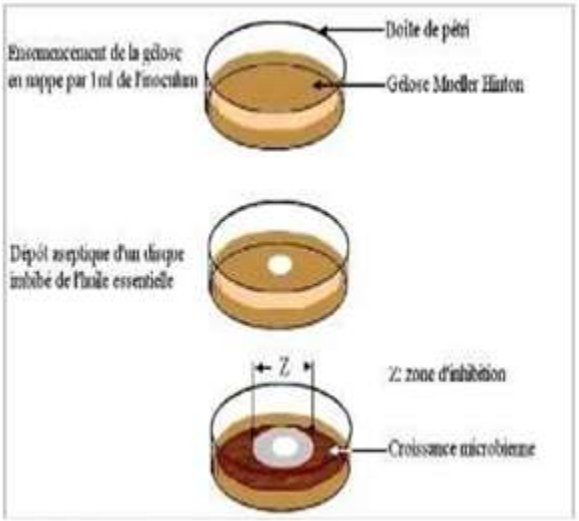

Figure 03. *Illustration of the aromatogram method*

Figure 04. *Preparation bench aromatogram.*

II.6. Antioxidant activity

II.6.1. Procedure

A solution of DPPH was prepared by solubilising 2.5 mg of DPPH in 100 ml of ethanol, a volume of 0.1 ml of each sample and control was mixed with 3.9 ml of DPPH, after incubation for 30 min in the dark and at room temperature, the absorbances were measured at 517 nm against the corresponding blank. The results were expressed as the average of three separate measurements ± standard deviation, and expressed as a percentage of the anti-radical activity using the following formula:

%AAR= D.O control - D.O sample/ D.O control x100

Figure 05. *DPPH and HE after 30 minutes of incubation*

II.7. Concentration of essential oils in polyphones

The determination of total polyphenols is carried out by the Folin-Ciocalteu method in order to quantify the content of total polyphenols. The reagent consists of a mixture of phosphotungstic acid (H3 PW12 O40) and phosphomolybdic acid (H3 PMo12 O40) of yellow colour. It is reduced, during the oxidation of the phenols, to a mixture of blue oxides of tungsten (W8O23) and molybdenum (Mo8O23) (Ribéreau-Gayon, 1968). The blue colour produced has a maximum absorption at around 760 nm. It is proportional to the quantity of polyphones present in the extracts.

II.7.1. Procedure

For the determination of polyphones, take 0.5 ml of each sample and the control (10ul diluted in 10 ml of distilled water), and add 5 ml of distilled water and 1 ml of Folin-Ciocalteu, after 3 minutes of rest 1 ml of sodium carbonate (10%) is added. The mixture is then homogenised and incubated at room temperature in the dark for 1 hour. After this time, the absorbance is determined at 760 nm using a spectrophotometer.

The determination of the total polyphenol concentration is carried out on the basis of a calibration curve y=ax+b, prepared under the same conditions, from a series of dilutions of Gallic acid.

The concentrations of total polyphenols are calculated from the regression equation of the calibration range established with gallic acid (y=0.99x + 0.107). These results provided estimates of the amounts of total polyphenols contained in the essential oil samples. Each test was repeated three times.

Figure 06. *the change of the yellow color to blue after the addition of HEs*

III. Results

III.1. The extraction of essential oils of Saccocalyx satureioides dried for 8 days from the two sites of Djelfa

The HEs of Zaafrane extracted by hydrodistillation and microwave, and Ain chouhada extracted by hydrodistillation gave a good yield of essential oil with a light yellow color and a strong and pleasant odor; then the essential oil of Zaafrane dried for 1 year with the same color and odor not so strong, after that of M'sila with a strong odor and a darker color. On the other hand the yield is 0% for the roots and the arms. So the determination of the yields of essential oils of Saccocalyx satureioides showed a good profitability (Zaafrane by hydrodistillation: 4.78%, Ain chouhada by hydrodistillation: 4.56%, Zaafrane after 1 year of drying by hydrodistillation: 2.56%, and 2.09% for M'sila by hydrodistillation; and for Zaafrane by microwave: 3.98% zafrane (Figure 07).

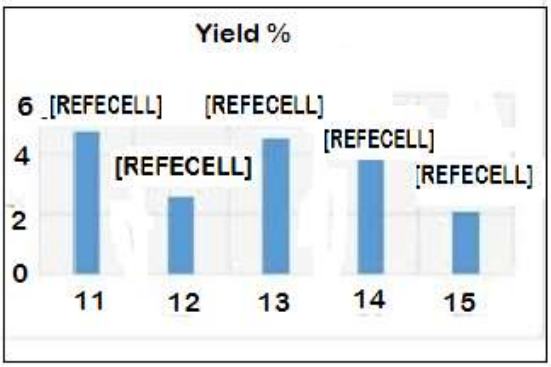

Figure 07. *The percentage yield of essential oils of Saccocalyx satureioides*

III.2. Chemical composition of the two populations of Saccocalyx satureioides by GC/MS

This chemical analysis revealed a large number of constituents of Saccocalyx satureioides from the two regions of the wilaya of Djelfa (Zaafrane and

Ain chouhada, of which we were able to identify 37 constituents, Among these constituents, Among these constituents, three are the most dominant, Thymol, a-terpineol, Borneol, (Figure 11), and four main chemical groups for the two sites, oxygenated monoterpenes, hydrocarbon monoterpenes, oxygenated sesquiterpenes, hydrocarbon sesquiterpenes (Figure 12).

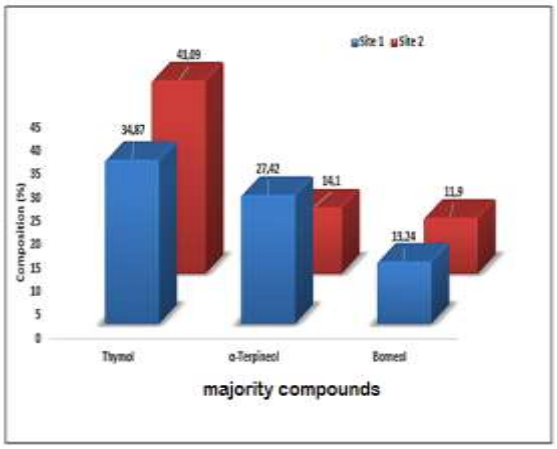

Figure 08. *Major constituents of the essential oil Saccocalyx satureioides from the Djelfa region.*

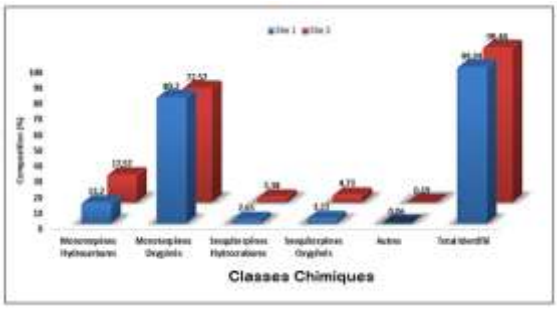

Figure 09. *Contents of chemical classes composing the EOs of the two populations of S.s.*

III.3. Evaluation of the antimicrobial activity of Saccocalyx satureioides essential oils

Essential oils are tested on four bacteria: Escherichia coli, Staphylococcus aurous, Pseudomonas aeruginosa, Bacillus subtilis, and a fungus: Candida albicans, the results show that these essential oils have an antibacterial and antifungal activity on all the strains tested, the thresholds of their power are between 10 and 28 mm.

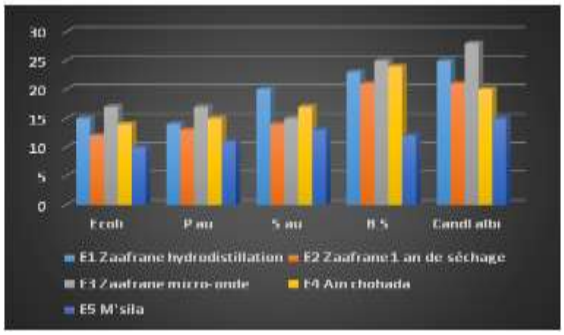

Figure10. *Antimicrobial activities of essential oils of Saccocalyx satureioides in mm*

*The diameters of the discs (6mm) are included in the measurements of the diameters of the zone of inhibition.

E1: Zaafran by hydrodistillation
E2: Zaafran after 1 year of drying by hydrodistillation
E3: Zaafran by microwave
E4 : Ain chouhada by hydrodistillation
E5 : M'sila by hydrodistillation.

III.4. Antioxidant activity of essential oils and their polyphenol concentrations

The results of the antioxidant activity revealed that all the essential oils of Saccocalyx satureioides have a significant antiradical activity (AAR) and this depends on the factors tested as well as their polyphenol compositions.

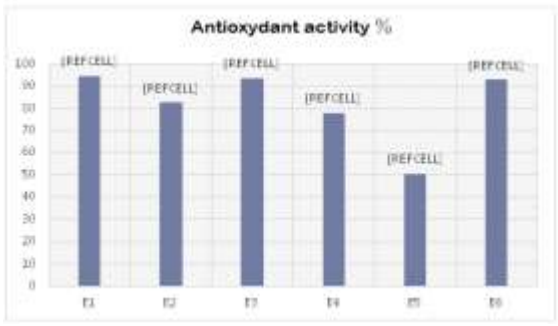

Figure 11. *Antioxidant activities of essential oils of Saccocalyx satureioides.*

E1: Zaafran by hydrodistillation
E2 : Zaafran after 1 year of drying by hydrodistillation
E3 : Zaafran by microwave
E4 : Ain chouhada by hydrodistillation
E5 : M'sila by hydrodistillation
E6 : Gallic acid

The amount obtained in polyphenols contained in the essential oils is presented in Figure 12.

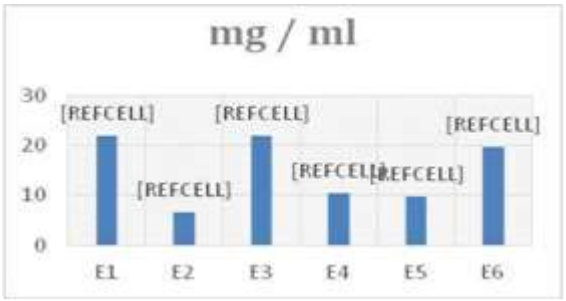

Figure 12. *Polyphenol concentration of essential oils of Saccocalyx satureoides*

IV. Discussion

IV.1. Yield

The results show a difference of 0.58% for the altitude factor, of which 4.78% for Zaafrane (875 m altitude) and 3.98% for Ain Chouhada (1231 m altitude).

For the origin of the plant between M'sila and Djelfa, the difference is 2.69%, a very important value and this is due to the change of the environment and therefore changes of climate and soil.

The yield of the essential oil of Saccocalyx satureioides is inversely proportional to the duration of drying of the plant, the difference in yield of the oil of a plant dried for one year and that dried for 8 days is 2.22%, which means that the essential oil decreases if the drying period is prolonged.

The extraction method by hydrodistillation and microwave gives a difference of 0.22% in yield, a low value but we found that the extraction method can also change the yield of the essential oil.

Among the organs used, we can directly detect that the leaves of Saccocalyx satureioides are rich in essential oil, unlike the roots and arms.

The altitude, the origin of the plant, the drying period, the extraction method and the organ are factors that can directly or indirectly influence the yield of essential oil to a greater or lesser extent.

IV.2. Chemical composition of Saccocalyx satureioides of two regions obtained by GC/MS

Chemical analysis by GPC yielded a range of constituents for both samples of Saccocalyx satureioides.

The combination of volatile compounds of this species is variable in terms of diversity and concentration.

In the essential oils obtained, 37 compounds were identified, representing 99.34% of the total chemical composition for Zaafrane and 98.44% for Ain chouhada.

Table 1. *Chemical composition of S. satureioides in essential oils from the two regions of Zaafrane and Ainchouhada*

Constituants[a]	***IRC[b]***	***IRA[c]***	***Composition (%)***	
			Site 1	***Site 2***
1- α-Thujene	*923*	*924*	*0,45*	*0,76*
2- α-Pinene	*929*	*932*	*1,14*	*1,88*
3- Camphene	***945***	***946***	***2,01***	***3,03***
4- Sabinene	*969*	*969*	*0,63*	*1,1*
5- β-Pinene	*974*	*974*	*0,24*	*0,31*
6- 3-Octanone	*981*	*979*	*tr*	*0,19*
7- Myrcene	*985*	*988*	*0,76*	*1,06*
8- α-Phellandrene	*1005*	*1002*	*tr*	*0,1*
9- α-Terpipene	*1014*	*1015*	*0,58*	*0,8*

10-p-Cymene	***1022***	***1020***	***3,12***	***3,69***
11-Limonene	*1025*	*1024*	*0,9*	*1,37*
12-γ-Terpinene	***1054***	***1054***	***3,08***	***3,26***
13-cis-Sabinenehydrate	*1068*	*1065*	*0,21*	*0,24*
14-α-Terpinolene	*1083*	*1083*	*0,23*	*0,21*
15-pLinalool	*1098*	*1095*	*0,64*	*0,74*
16-α-Campholenal	*1123*	*1122*	*0,13*	*0,18*
17-Camphor	*1142*	*1141*	*0,1*	*0,12*
18-Borneol	***1169***	***1165***	***13,24***	***11,9***
19-Terpinene-4-ol	*1178*	*1174*	*2,25*	*1,95*
20-α-Terpineol	***1195***	***1186***	***27,42***	***14,1***
21-Bornylacetate	*1278*	*1279*	*tr*	*0,12*
22-Thymol	***1299***	***1289***	***34,87***	***41,09***
23-Thymylacetate	*1342*	*1355*	*1,18*	*1,9*
24-Carvacrolacetate	*1362*	*1370*	*tr*	*0,23*
25-α-Gurjunene	*1399*	*1409*	*0,15*	*0,19*
26-β-Caryophyllene	*1412*	*1412*	*1,19*	*1,62*
27-α-Humulene	*1449*	*1452*	*0,16*	*0,25*
28-α-Patchoulene	*1453*	*1454*	*0,14*	*0,21*
29-Bicyclogermacrene	*1489*	*1500*	*0,94*	*0,97*
30-δ-Cadinene	*1520*	*1522*	*tr*	*0,14*
31-Germacrene D-4-ol	*1577*	*1574*	*0,37*	*0,61*
32-Globulol	*1583*	*1590*	*0,13*	*0,35*
33-Ledol	*1602*	*1602*	*0,07*	*0,48*
34-Cubenol	*1645*	*1645*	*0,15*	*0,15*
35-t-Cadinol	*1647*	*1652*	*0,14*	-
36-α-Cadinol	*1657*	*1652*	*0,41*	*0,16*
37-Shyobunol	*1694*	*1688*	*1,96*	*2,98*
Monoterpènes Hydrocarbures			*13,2*	*17,57*
Monoterpènes Oxygénés			*80,2*	*72,57*
Sesquiterpènes Hydrocarbures			*2,65*	*3,38*
Sesquiterpènes Oxygénés			*3,23*	*4,73*
Autres			*0,06*	*0,19*
Total Identifié			*99,34*	*98,44*

a) RRI = Retention indices calculated on the non-polar RRI = Retention indices reported by Adams (2007). Site 1 = Zaafrane, Site 2 = Ain chouhada. d) Components first reported in S. satureioides essential oil. tr = trace (< 0.1%). Majority compounds are in bold. %: relative abundance of compounds measured on apolar column.

Table 2. *Majority of chemical compounds of S. satureioides in the essential oils of the two regions of Zaafrane and Ainchouhada*

component	*% in oil extracted from the Ain chouhada plant*	*% in oil extracted from the Ain zaafrane plant*
Oxygenated Monoterpenes	*80.2%* *- Thymol=36.42%* *- α-Terpineol=28.97%* *- Borneol=14.79%*	*72.57%* *- Thymol=41.09%;* *- α-Terpineol=14.1%* *- Borneol=11.9%*
Monoterpenes Hydrocarbons	*17.57%* *- p-Cymene=6.48%* *- γ-Terpinene=6.08%* *- Camphene=5.01%*	*13.2%* *- p-Cymene=4.91%* *- γ -Terpinene=4.26%* *- Camphene=4.03%*
Oxygenated sesquiterpenes	*4.73%*	*3.23%*
Sesquiterpenes Hydrocarbons	*3.38%*	*2.65%*
Other constituents	*0.19%*	*0.06%*

It can be seen that the class of oxygenated monoterpenes is the most dominant in the essential oils, with a percentage of 80.2% for the site of Zaafrane (Thymol=41.09%; α-Terpineol=14.1% and Borneol=11.9%); and 72.57% for the site of Ain chouhada (Thymol=34.87%;α-Terpineol=27.42% and Borneol=13.24%) Among the 37 constituents identified, we were able to determine the 6 most dominant constituents which are Thymol with a percentage of 34.87% for Zaafrane 41.09% for Ain Chouhada

α-Terpineol with a percentage of 27.42% for Zaafrane and 14.1% for Ain chouhada Borneol with a percentage of 13.24% for Zaafrane and 11.9% for Ain Chouhada the p-Cymeneand 3.12% for Zaafrane 3.69% for Ain chouhada the γ-Terpinene 3.08% for Zaafrane 3.26% for Ain chouhada Camphene with a percentage of 2.01% for Zaafrane and 3.03% for Ain chouhada.
The 37 identified constituents are grouped into 4 groups and one group containing other constituents:
- Monoterpenes Hydrocarbons,
- Oxygenated monoterpenes,
- Sesquiterpenes Hydrocarbons,
- Oxygenated sesquiterpenes,
- Others

From the figure, we can see that the class of oxygenated monoterpenes is the most dominant in the essential oil of Saccocalyx satureioides, with a percentage of 80.2% for site 1 (Thymol=41.09%; α-Terpineol=14.1% and Borneol=11.9%); 72.57% for site 2 (Thymol=34.87%;α-Terpineol=27.42% and Borneol=13.24%), thus the essential oil of Zaafrane has more oxygenated monoterpenes than that of Ain chouhada.

The Hydrocarbon Monoterpenes class follows with a percentage of 13.2% for site 1 (p-Cymene=3.69%, γ-Terpinene=3.26% and Camphene=3.03%); and 17.57% for site 2 (p-Cymene=3.12%, γ-Terpinene=3.08% and Camphene=2.01%), so this class is dominant in Ain chouhada essential oil.

Oxygenated Sesquiterpenes with a percentage of 3.23% for site 1 and 4.73% for site 2, so it is dominant in the essential oil of Ain chouhada. And finally we have the class of Sesquiterpenes Hydrocarbons with a percentage of 2.65% for site 1 and 3.38 for site 2, so it is dominant in the essential oil of Ain chouhada. And a class that presents the other constituents we have 0.06 for Zaafrane and 0.19 for Ain chouhada.

In summary: 99.34% of the constituents for the site of Zaafrane and 98.44% for the site of Ain chouhada.

According to Daniela M. Biondi's dissertation on Saccocalyx satureioides, the number of constituents identified is 41, which are classified into 3 groups: Oxygenated Monoterpenes (76.9%), Monoterpenes-Hydrocarbons (16.8%), and Sesquiterpenes (2.7%). Our results are similar to the results of this study, except that we have separated the Sesquiterpenes into 2 classes.

IV.3. Evaluation of antimicrobial activities of essential oils of Saccocalyx satureioides

All essential oils were tested on four bacteria.

If we compare the measurements of the zone of inhibition of the tested samples, we notice that the essential oil of Zaafrane extracted by microwave is the most powerful compared to the others, (on 3 bacteria, and the fungus "15-28mm"); then we have the essential oil of Zaafrane extracted by hydrodistillation (1 bacteria and the fungus"14-25mm"); and then Ain chouhada by hydrodistillation "14-24 mm"; after that of Zaafrane

dried during 1 year (between 12-21); and lastly that of M'sila (10- 15), these last two are less active, and that of Zaafrane extracted by microwave is the most effective.

Practically the essential oil of Saccocalyx satureioides has more antifungal activity than antibacterial activity, except in the case of Ain chouhada.

The antimicrobial power of the essential oil of Saccocalyx satureioides changes with the factors tested, altitude, origin of the plant, drying period, extraction method. It is postulated that the different components of essential oils show a difference in the degree of antimicrobial activity against G+ and G- bacteria [12].

Several works, including those of (Hammer et al. 1999; Souza et al. 2006; Derwich et al. 2010 and Bari et al. 2010), have confirmed the high resistance of G- bacteria compared to G+, this is due to the presence of a lipopoly-saccharide layer (LPS) in G- bacteria that could function as an effective barrier (Inouye et al. 2001 and Bagamboula et al. 2004). And this is effectively implied in the case of essential oils of Saccocalyx satureioides, E.coli and pseudomonas aeruginosa (Gram -)(between10-17mm), they resisted the essential oils tested compared to the other two bacteria (12-25) and fungus(15-28mm).

According to Cosention et al. (1999) and Gulfraz et al. (2008), the antimicrobial activity of all essential oil is assigned to terpenoids and phenolic compounds. Based on GC/MS, the essential oil of Saccocalyx satureioides is dominated by terpene compounds.

The antimicrobial activity of essential oils could largely be associated with its main constituents: hydrocarbon monoterpenes that have shown antimicrobial properties [13].

According to the GC/MS results, monoterpenes hydrocarbons are important of 13.2% for Zaafrane and 17.57% for Ain chouhada.

Moreover, the positive germs tested justify the popular use of this plant and confirm its therapeutic anti-diarrheal activity: S.aureus and Bacillus are fungus, and their antimicrobial potencies were measured by the diameters of the inhibition zones in mm.

From the results, it can be seen that all essential oils of Saccocalyx satureioides have antibacterial and antifungal activity on all strains with zone of inhibition between 10 and 28 mm.

Figure13. *photos of antimicrobial activities of essential oils of Saccocalyx satyreioides*

IV.4. Evaluation of antioxidant activity
***Antioxidant**

The antioxidant activity of essential oils ranges from 94.44% to 50.60%, which means the effectiveness of this essential oil, this change in AAR may be related to the factors tested, and also to the polyphenol composition, and in general the chemical composition of essential oils.

The antioxidant activity of the essential oils of Zaafrane extracted by hydrodistillation and microwave are the most powerful (94.44% and 93.65%), then that of Ain chouhada (82.53%), the altitude and the method of extraction did not change much their AAR, but the variability is there and therefore they have an impact on the composition of essential oils; and for the period of drying for 1 year the AAR is the lowest (50. 60%), so the effectiveness decreases if we increase the drying period, the AAR of the essential oil of M'sila (78.03%) confirms that the change of the environment, climate and soil can play on the chemotype of the plant and therefore also on the AAR. I took the gallic acid as a reference (92.85%), in comparison with the tested samples; it was found that the essential oil of Zaafrane is more effective than the gallic acid.

The results of the antioxidant activity revealed that all the essential oils of Saccocalyx satureioides have a significant antiradical activity (AAR) and

this depends on the factors tested as well as their compositions in polyphenols.

IV.5. The polyphenol content and its relation to the antioxidant activity

Polyphenols are the most abundant natural antioxidants in our diet, according to estimates we consume the equivalent of one gram per day, ten times more than vitamin C and one hundred times more than carotenoids and vitamin E [23]. These compounds are still of interest to manufacturers since their contents and their compositions precisely influence the organoleptic quality (color, astringency, bitterness) of the fruits transformed into juice, wine, etc. [24].

Chemical compounds are widely present in the plant kingdom, and most have their phenols of antioxidant power, thanks to their hydroxyl groups. If we compare the antioxidant activity of essential oils with their polyphenol concentrations, we find that essential oils by hydrodistillation and microwave have the most powerful antioxidant activities, and in parallel high polyphenol concentrations (21.95 and 21.93mg / ml) compared to gallic acid (19.73mg / ml), and for the essential oil of Saccocalyx satureioides dried for 1 year had the lowest antioxidant activity with a concentration of polyphenols the lowest also (6.6mg / ml). Then on an Ain chouhada and M'sila (10.44 and 9.69 mg / ml) which is in the middle.

V. Conclusion

The present work is devoted to the determination of the yield, chemical composition, antibacterial, antifungal, antioxidant properties, and polyphenol concentration of essential oils extracted from Saccocalyx satureioides harvested in two wilayas namely Djelfa and M'sila according to several factors, the origin of the plant, the altitude, the method of extraction, the period of drying, and the organ.

The determination of the yields showed a good profitability in volatile oil in Saccocalyx satureioides for the tested factors, (Zaafrane by distillation: 4.78%, Zaafrane by microwave: 4.56%, Ain chouhada by hydrodistillation: 3.98%, Zaafrane 1 year of drying by hydrodistillation: 2.56%, and finally M'silahydrodistillation: 2.09%. This variation may be due to the chemotype of each individual. On the other hand 0% for the roots and arms, this means the absence of essential oil in these organs.

The chemical composition by GC/MS of the essential oils of the two stations of the wilaya of Djelfa, determined four main groups: oxygenated monoterpenes, hydrocarbon monoterpenes, oxygenated sesquiterpenes, hydrocarbon sesquiterpenes.and six dominant constituents: Thymol, a-terpineol, Borneol, p-Cymene, y-Terpinene and finally Camphene. The two sites present different values, and thus two different chemical compositions, and this comes back to the altitude (Zaafrane: 857m, and Ain chouhada: 1231m), this difference caused a variation in the chemical composition of Saccocalyx satyreioides, and subsequently variations in their antibacterial, antifungal, antioxidant activities, and also their concentrations in polyphenols. This may explain the variation in the results for the other factors, but this remains to be confirmed.

The antibacterial and antifungal activity obtained by the aromatogram in this study, show that the essential oils of Saccocalyx satureioides have a significant inhibitory activity on the four bacteria and fungus tested (Zaafrane by hydrodistillation: 14-25mm, Zaafrane by microwave: 15-28mm, Ain chouhada by hydrodistillation: 14-24mm, Zaafrane 1 year of drying: 12-21mm, and finally M'sila: 10-15mm. Practically the antifungal activity (15-28mm) of this essential oil is more active than the antibacterial activity (10-25mm). So this essential oil has more power on the fungus than on the bacteria. And the essential oil of Zaafrane extracted by microwave is the most effective on practically all the tested strains.

The relationship between the antioxidant activity of the essential oils and their polyphenol concentrations is almost perfect, so the antioxidant activity increases with the polyphenol concentration, which confirms that polyphenols are responsible for the antioxidant activity of the essential oils of Saccocalyx satureioides. (The antioxidant activity for Zaafrane by hydrodistillation is 94.44% with a polyphenol concentration of 21.95 mg/ml, Zaafrane by microwave: 93.65% with 21.93mg/ml, Ain chouhada by hydrodistillation: 82. 53% with 10.44mg/ml, Zaafrane 1 year of drying: 50.6% with 6.6mg/ml, M'sila: 78.03% with 9.69mg/ml, and finally for the reference which is the gallic acid: 92.5% with 19.71mg/ml

The yield, chemical composition, antibacterial, antifungal, antioxidant activities, and polyphenol concentration of essential oils of Saccocalyx satureioides vary from one individual to another, this variability can be related to extrinsic and intrinsic factors. The same plant, growing in different places, with different altitude, climate, pedology, etc., different drying period, different organ used, and a different method of extraction, all this implies a variation in chemotype, yield, and subsequently in the activities of its essential oil.

Finally, all the results obtained are only a first step in the search for biologically active natural source substances, further tests will be necessary to

confirm the multiple biological activities of this essential oil as well as the factors that increase its effectiveness.

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Please cite this Article as:
Draoui Y., Aksas H., Amghar F., Extraction and valorization of active principles of Saccocalyx satureioides collected in two wilayas of Algeria., ***Algerian J. Env. Sc. Technology***, **8:2 (2022) 2495-2505**