International Journal of Scientific Research & Engineering Technology (IJSET)
Vol.10 pp.1-9

Extraction and valorization of the active ingredients of Saccocalyx satureioides harvested in two stations of the province of Djelfa

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***ABSTRACT*:**

This study was conducted to analyze the yield, chemical composition and antibacterial and antifungal activities of essential oils of *Saccocalyx satureioides* harvested in two stations of the province of Djelfa under different ecological conditions. The essential oil is extracted by steam distillation using Clevenger-type extraction device. The yield was 0.87% for the harvested plant to Zaafrane and 1.32% for the Ain Chouhada. Oil *Saccocalyx satureioides* exhibits antibacterial activity on bacteria: Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, and antifungal activity on the fungus Aspergillus Niger. The results show that the antibacterial activity of the essential oil of Ain chouhada (12-20mm) is more important than that of Zaafrane (10-17mm), with a significant antibacterial activity of the antifungal activity. The chemical analysis of essential oils *Saccocalyx satureioides* GC-MS revealed the presence of constituents dominated by: thymol, α-Terpineol, Borneol, p-Cymene, γ-Terpinene, and finally we have the Camphene.

Keywords*:* *Saccocalyx satureioides*, essential oils, antimicrobial activity, antifungal activity, Djelfa.

ISSN 1737-9296

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Introduction

Algeria is considered among the countries known for their taxonomic diversity due to its privileged geographical position and its spread between the Mediterranean and sub-Saharan Africa. As well as the Algerian steppe presents a remarkable interest from the point of view of its specific vegetal richness. (Ozenda, 1977). Among this floristic richness we are interested in the plant species "Saccocalyx Satureioides", harvested in two communes of high Algerian plains namely, Zaafrane and Ain chouhada which are located in the wilaya of DJELFA, in order to carry out the Extraction of the essential oils of the two sites by hydrodistillation, the identification of the chemical composition by (GC-MS) of the two extracted essential oils, and finally to test their antibacterial and antifungal activities, to see if the composition chemical varies with environmental factors.

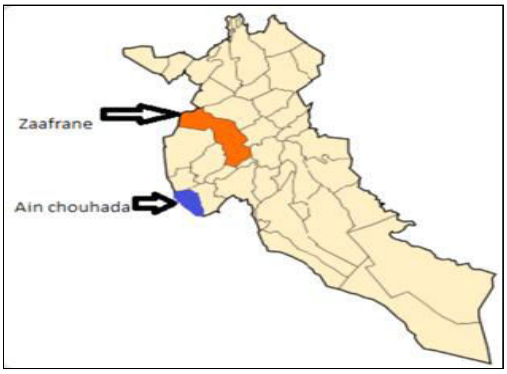

Figure 01: Location of the two municipalities in Djelfa wilaya

Figure 02: Saccocalyx saturated

METHODOLOGY :

Drying:

After harvest of saturated Saccocalyx from both sites by mid-April 2016. The stems and leaves were dried in the dark and at room temperature for 8 days.

Hydrodistillation :

It is a method of extraction of essential oils carried out at the level of laboratory of ecology of the department of process engineering (UMBB) by a distillation apparatus of the modified Clevenger type, whose role is to drive the volatile compounds of the natural products with water vapor. It makes it possible to extract the maximum of the organic compounds (essences) contained in the plants.

We introduce into a 2-liter pyrex glass flask, 40 g of plant material, then we add a volume of distilled water that corresponds to 2/3 of the capacity of the flask. Then we adapt to the balloon condensing device of the vapor (refrigerant) whose temperature does not exceed 23 ° C, we supply it with water and the heating with a balloon heater, and We close the balloon with a thermometer to check the temperature. After 15 to 20 minutes, when the temperature reaches 90 ° C, the water begins to boil causing steam and essential oil that will liquefy in the cooling column. Finally we collected a liquid composed of two phases: Aqueous phase and oily phase, which is the distillate. the decantation is done automatically, at the end of the extraction we recover our essential oil in a bottle of brown glass, sterile, well closed and kept at a temperature of 0 to 4 ° C, to preserve it from air and the light, The duration of extraction is 2 to 4 hours maximum and the operation is repeated several times (3 times maximum).

Without forgetting to note its weight with a scale of precision to be able to calculate the yield which is given by the following formula:

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$$Rd = (M')/M \times 100$$

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).

Figure 03: Installation of a modified clevenger type hydrodistillator, made at the ecology laboratory (UMBB).

Gas chromatography coupled with mass spectrometry:

GC / MS is a separation analysis method that applies to gaseous compounds or compounds that can be vaporized by heating without decomposition (Arpino, et al., 1995). Made in France, it is the separation technique most used in the field of essential oils, because it allows the individualization of constituents from samples of the order of milligram or microgram.

Aromatogram:

The evaluation of the antimicrobial activity of the essential oil of Saccocalyx satureioides was made on 4 bacteria and a mushroom recovered from the microbiology team of the laboratory VALCOR of the UMBB. 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 matter.

It is an agar diffusion method that aims to qualitatively evaluate the "in 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. (**Remmel et al., 1993**).

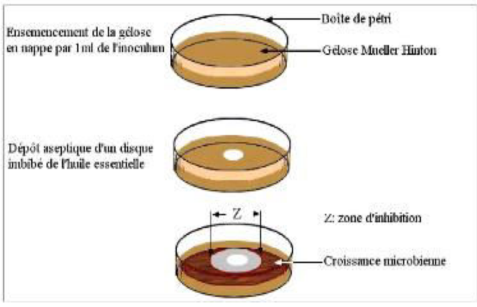

Figure 04: Illustration of the aromatogram method

Figure 05: Preparation bench.

RESULTS:

Hydrodistillation :

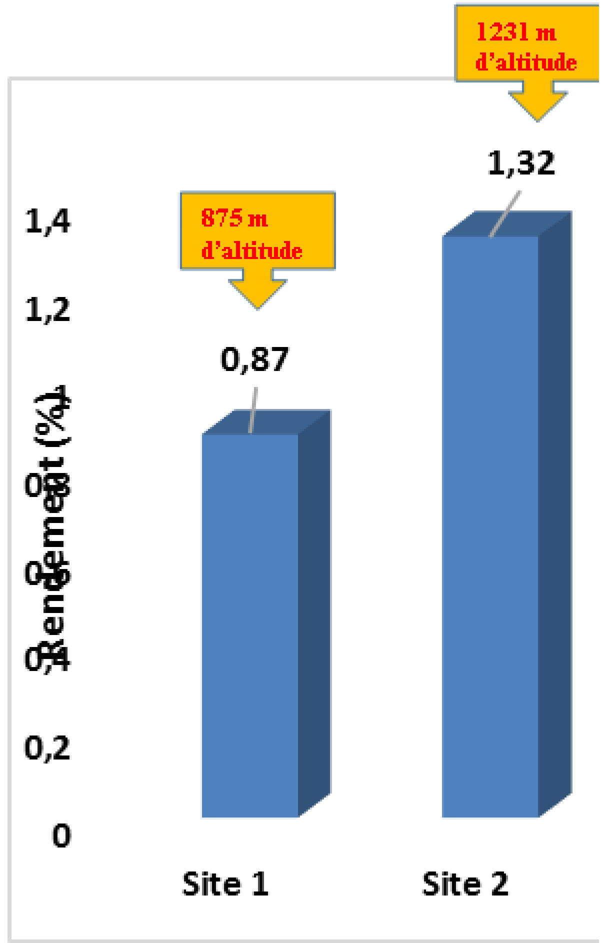

Figure 06: Comparative yield in essential oils of the two populations of S. satureioides.

The determination of hydrodistillation yields of essential oils showed a good yield of volatile oil in Saccocalyx satureioides (0.87% for the Zaafrane sample and 1.32% for that of Ain chouhada).

Comparative chemical composition of the essential oils of the two S. satureioides populations:

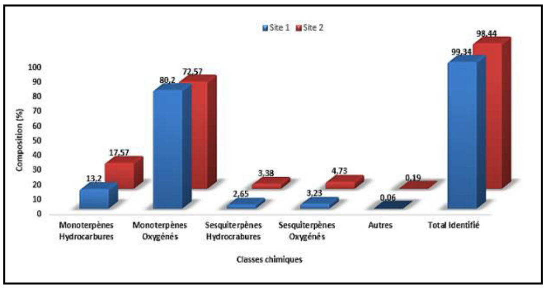

Figure 07: Content of the chemical classes composing the HEs of the two populations of S. satureoides.

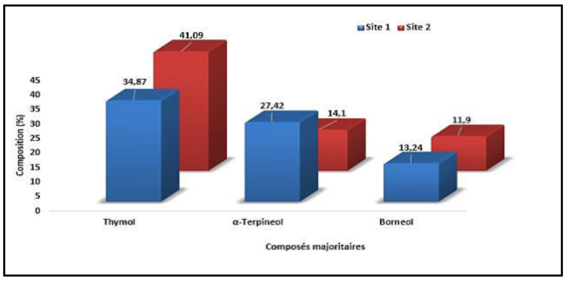

Figure 08: Content of three major constituents of HEs of two populations of S. satureoides.

The results of the chemical analysis of the composition of essential oils by (GC / MS) determined four main chemical groups for the two sites: Monoterpenes Hydrocarbons, Oxygenated Monoterpenes, Sesquiterpenes Hydrocarbons, and Oxygenated Sesquiterpenes.

And then This analysis made it possible to isolate and identify 37 compounds for the two stations representing 99.34% for Zaafrane of HE; and 98,44% for Ain chouhada.

The most dominant constituents are: Thymol with a percentage of 34.87% for Zaafrane and 41.09% for Ain chouhada; then there is α-Terpineol with a percentage of 27.42% for Zaafrane and 14.1% for Ain chouhada; and finally we have Borneol with a percentage of 13.24% for Zaafrane and 11.9% for Ain chouhada.

Evaluation of the antimicrobial activity of the essential oil:

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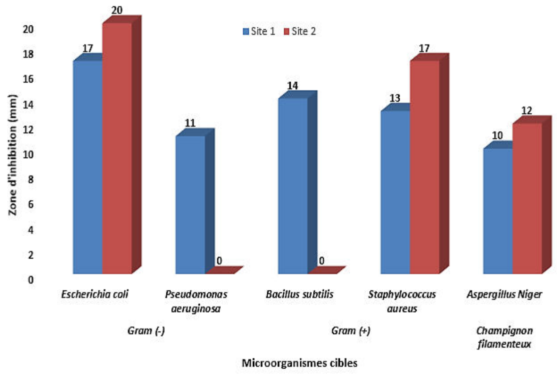

Figure 08: Antimicrobial activities (zone of inhibition in mm) of the essential oils of the two populations of S. satureoides.

The antibacterial and antifungal activity obtained by the aromatogram show that the essential oil of Saccocalyx satureioides has a significant inhibitory activity on the four bacteria: E. coli, Pseudomonas, Staphylococcus, Bacillus, and on the fungus: Aspergillus, with some variation for both sites. The results show us that the antibacterial activity of the Ain chouhada essential oil (between 12-20mm) is greater than that of Zaafrane (10-17mm), with a significant antibacterial activity of the antifungal activity.

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 matter.

DISCUSSION:

Hydrodistillation :

The extraction of the essential oil from the plant at both sites by hydrodistillation gave a good yield of HE with a light yellow color and a strong and pleasant odor.

The difference between the yields of the two sites is 0.45%, it is an important value that can come back to several factors that differ from one medium to another.

This difference in yield between the two sites can be attributed to many factors such as the stage of growth, pedoclimatic and edaphic conditions of the region, extraction technique (Fellah, 2006) plant genetics, organ of the plant used, the degree of freshness, sunshine, rainfall, these factors among others that may have a direct impact on HE performance (Vekiari et al, 2002).

The yield of the essential oil depends on several factors such as species, altitude, geography, harvest period, cultural practices, extraction technique, etc. (Silano and Delbo 2008, Marzoukia et al., 2009, Olle and Bender 2010).

Comparative chemical composition of the essential oils of the two S. satureioides populations:

It is found that the class of the Oxygenated Monoterpenes is the most dominant in the essential oil of Saccocalyx satureioides, with a percentage of 80.2% for the site 1 (Thymol = 41.09%, α-Terpineol = 14.1% and Borneol = 11.9%); and 72.57% for site 2 (Thymol = 34.87%, α-Terpineol = 27.42% and Borneol = 13.24%), so the essential oil of Zaafrane has more Oxygenated Monoterpenes than that of Ain chouhada.

The class Monoterpenes Hydrocarbons 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%), therefore this class is more dominant in the essential oil of Ain chouhada. After the class of oxygenated sesquiterpenes with a percentage of 3.23% for site 1 and 4.73% for site 2 therefore 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, therefore 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.

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We notice a certain variability of the constituents containing the essential oil of the two saturated Saccocalyx populations, these observed differences can be related to the different chemotypes, time of harvest, altitude, soil, temperature, rainfall, humidity, sunshine, neighboring plant populations, etc.

In addition, broadening the comparison within the framework of the Lamiaceae family.

The work of many authors has shown that plants react to the surrounding environment and that during their lifetime, the chemical composition of their metabolites could evolve. Extracts of plants, essential oils are very fluctuating in their composition , which can vary according to the geographical and climatic conditions, the cultivation ground of the plant, the year of culture (sunshine, hygrometry, etc.), the cultivation mode, the harvesting period, the individual or the organ considered, the extraction method, etc. (**Bignell and Dunlop, 1994**).

Diversity according to the plant organ: For a species whose different organs may contain an essential oil, the chemical composition of it may vary from one organ to another. (Richard and Loo, 1992). Influence of the harvest period: The proportion of different constituents of the essence of a given species can vary considerably during its development. The maturity or phenological state of the plant at the time of harvest are difficult to verify and control (Derbesy, 1997).

Indeed, on the same stem, the leaves or the flowers do not appear simultaneously and according to their age, do not have the same composition (Touche, 1997). This has been demonstrated in particular on mint where differences in chemical composition have been observed between the edges and the center of the sheet.

Existence of chemical varieties or "chemotypes": A plant species perfectly defined botanically can give species whose chemical composition is different according to the individuals, (Bruneton J., 1993).

For some authors, chemotypes constitute, within the species, chemical varieties each possessing a particular enzymatic equipment, determined genetically and which directs the biosynthesis towards the preferential formation of a precise constituent (Pellecuer, 1982).

The way in which extractions are carried out (time of operations, packing and crushing of vegetable matter, number of washes, etc.) can affect the composition and the organoleptic characteristics of the essence.

The flowering tops and leaves should be harvested before flowering, because according to Salle and Pelletier (1991), after flowering, 70% of essential oils evaporate in the air, however, the whole plant is usually harvested during flowering. A number of parameters play a vital role in the chemical composition of Essential Oil Plants, (Girre, 1979) and (Anton et al., 2005):

• Light: each plant has its requirements for light quality and photoperiodism, most often prefer direct sunlight for at least six to eight hours.

• Temperature: the optimal T ° is decisive for each plant.

• Fertilization: in general, the fertilization of the crop should be limited or even absent for dry scrubland plants (thyme, rosemary, savory).

• Soil: Most aromatic herbs require rich, loose, well-drained soil with neutral or slightly alkaline pH.

In fact, each plant species has its biological and ecological requirements, its own evolution and its reactions to the environment in which it lives. As the botanist FLAHAUT wrote: "Every species has its place in nature by laws to which man can not change anything" (Pellecuer, 1982).

Evaluation of the antimicrobial activity of the essential oil:

The evaluation of the antimicrobial activity of our essential oil at both sites was tested on 5 human pathogens, four bacteria: Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, as well as its antifungal activity on a fungus. : Aspergillus niger.

The antimicrobial activity of essential oils of Saccocalyx satureioides could largely be associated

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with its main constituents: Monoterpenes Hydrocarbons which have shown antimicrobial properties (Derwich et al, 2010).

From the results mentioned in the two tables, it is found that the saturated Saccocalyx essential oil has antibacterial and antifungal activity on all the strains tested. The thresholds of its antimicrobial power are between 10 and 20 mm for both sites.

All strains have been shown to be sensitive to this essential oil. But Ain chouhada (12-20 mm) saturated saccocalyx essential oil is much more active than Zaafrane (10-17 mm).

We note that Saccocalyx sematioides HE from both sites does not have a very good activity on the fungus Aspergillus niger (10-12 mm) compared to its activity on bacteria (11-20 mm). So its antibacterial activity is more active than its antifungal activity.

Several works including those of Hammer et al. (1999); Souza et al. (2006); Derwich et al. (2010); and Bari et al. (2010) confirmed the high resistance of G- bacteria to G +, which is equivalent to the presence of a lipopolysaccharide layer (LPS) in G- bacteria that could function as an effective barrier against bacteria (Inouye et al. 2001, Bagamboula et al., 2004). But this does not get involved effectively in the case of Saccocalyx satureioides, E. coli proved to be more sensitive although it is Gram-.

It is postulated that the different components of HEs show a difference in the degree of antimicrobial activity against G + and G- bacteria (Dorman and Deans, 2000).

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

The antimicrobial activity may also depend on the composition of the culture medium (Dorman and Deans, 2000).

The antimicrobial activity of essential oils of Saccocalyx satureioides could largely be associated with its main constituents: Monoterpenes Hydrocarbons that have shown antimicrobial properties (Derwich et al, 2010). According to the results of the GC/MS, the Monoterpenes Hydrocarbons are 13.2% for Zaafrane and 17.57% for Ain chouhada, so that is why the antimicrobial activity of Ain chouhada is more active than that of Zafrane.

In addition, the positive test organisms justify the popular use of this plant and confirm its anti-diarrheal therapeutic activity: S.aureus, E. coli and Bacillus are germs very often implicated in food poisoning.

Conclusion :

Determination of hydrodistillation yields of essential oils showed a good yield of volatile oil in Saccocalyx satureioides (0.87% for the Zaafrane sample and 1.32% for that of Ain chouhada). Yield increases with altitude.

The antibacterial and antifungal activity obtained by the aromatogram in this study, show that the essential oil of Saccocalyx satureioides exhibits significant inhibitory activity on the four bacteria: Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, and the fungus: Aspergillus, with some variation for both sites, and this may return to the chemical composition of the species.

The results show us that the antibacterial activity of the Ain chouhada essential oil (12-20mm) is greater than that of Zaafrane (10-17mm), with a significant antibacterial activity of the antifungal activity.

The yield, chemical composition and antimicrobial potency of essential oils vary from one individual to another. This variability may be related to extrinsic and intrinsic factors.

So we can say that Hes coming from the same plant but coming from different geographical zones, imply a variation of the yield in aromatic molecules as well as a variation of the nature of these molecules.

Finally, all the results obtained are only a first step in the search for biologically active natural source substances. Further tests will be needed to confirm the multiple biological activities of this essential oil as well as the factors that increase its effectiveness.

Bibliography:

ISSN 1737-9296

[1] : **Anton R., A. Lobstein, Teuscher F**., (2005). -Aromatic Plant: Spices, Aromates, Condiment And Essential Oil. Ed: Tec And Doc. Paris: 521.

[2] : **Arpino P., Provost A., Serpinet J., Tranchant J., Vergnol A., Witier P**., 1995. Practical handbook of gas chromatography, Masson, Paris.

[3] **: Bagamboula C.F., Uyttendaele M. and Debevere J**., 2004. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards Shigellasonnei and S. flexneri. Food Microbiology 21: pp. 33-42.

[4] : **Bari M.A., Islam W., Khan A.R. and Mandal A.**, 2010. -Antibacterial and antifungal activity of Solanum torvum (Solanaceae). Int. J. Agric. Biol., 12: pp. 386-390.

[5] : **BIGNELL C.M. and DUNLOP P.J.** (1994). -Volatile Leaf Oils Ofsorne South-Western and Southern Australian Species Ofthe Genus Eucalyptus. Part 1 Subgenussymphyomyrtus, Dumaria Section, Seriesincrassatae, Flavourfragr. 113-117.

[6] : **Bruneton J.**, 1993.-Pharmacognosy. Phytochemistry, Medicinal Plants. Tec. & Doc. Lavoisier, 2nd Edition, Paris. 915P.

[7] : Cosentino S., Tuberoso C.I.G., Pisano B., Satta M., Mascia V., Arzedi E. And Palmas F., 1999. -In Vitro Antimicrobial Activity And Chemical Composition Of Sardinian Thymus Essential Oils. Letters In Applied Microbiology 29, pp131-135.

[8] : DERBESY M. (1997). -Reproductibility of Industrial Natural Extracts, Pai:! Ums Cosmetics News, 132, p. 57-59.

[9] : Derwich E., Benziane Z. & Boukir A., 2010. -GC / MS Analysis And Antibacterial Activity Of The Essential Oil Of Menthapulegium Grown In Morocco. Res. J. Agric. & Biol. Sci., 6 (3): Pp. 191-198.

[10] : Dorman H.J.D. And Deans S.G., 2000. -Antimicrobial Agents From Plants: Antibacterial Activity Of Plant Volatile Oils. Journal of Applied Microbiology 88, pp. 308-316.

[11] : Fellah S, Romdhane M., Abderraba M., 2006. "Extraction and Study of Essential Oils of Salviaofficinalis.L Plucked in Two Different Regions of Tunisia" J.Soc.Alger.Chim., 2006, 16 (2), 193-202. Journal of the Algerian Society of Chemistry.

[12] : GIRRE, (1979). -New Guide of Old Natural Remedies. Ed: Rennes. La France. 314 P ISBN 2-85882-860-1.

[13] : Gulfraz M., Mehmood S., Minhas N., Jabeen N., Kausar R., Jabeen K. And Arshad G., 2008. -Composition And Antimicrobial Properties Of Essential Oil Of Foeniculum Vulgare. African Journal Of Biotechnology Vol. 7 (24), Pp.4364-4368.

[14] : K. Hammer, Carson C. F., & Riley T., 1999. -Antimicrobial Activity Of Essential Oils And Other Plant Extracts. Journal of Applied Microbiology Vol. 86, Issue 6: Pp.985-990.

[15] : Inouye, S., Tsuruoka, T., Uchida, K., Yamaguchi, H., 2001. -Microbiol. Immunol., 43, pp.201– 208. In Ahmad I., Aqil F. and Owais M. Modern Phytomedicine: Turning Medicinal Plants into Drugs. Ed. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, 405p.

[16]: Marzoukia H., Elaissib A., Khaldic A., Bouzidd S., Falconierie D., Marongiu B., Pirasa A. and Porcedda S., 2009. -Seasonal and geographical variation of Laurusnobilis L. essential oil from Tunisia. The Open Natural Products Journal, Vol. 2; pp. 86-91.

[17] : Olle M. and Bender I., 2010. -The content of oils in umbelliferous crops and its formation. AgronomyResearch 8 (3), pp.687-696.

[18] : Ozenda P., 1977. -Florida Sahara. Paris, C.N.R.S., 622 P.

[19]: Pellecuer J. (1982). - From the Plant to the Medicine: Medicinal Plants and Popular Medicine in Bas Languedoc, Document Published by Odac, Supplement to Studies on the Hérault, 13 (3), P. 29-35.

[20]: Remmal A., Tantaoui-Elaraki A., Bouchikhi T. & Ettayebi M., 1993. -Improved Method For The Determination Of Antimicrobial Activity Of Essential Oils In Agar Medium. J. Essent. Oil. Res. 5: P.179-184.

ISSN 1737-9296

[21]: Richard H. And Loo A. (1992). -Nature, Origin And Propriety Of Spices And Raw Aromates: In Richard H., Aromatians & Spices, Tec. & Doc. Lavoisier, Paris, P. 17-69 and P. 213-238.

[22]: Salle J.L. and Pelletier J., 1991. -Essential oils, aromatherapy synthesis and introduction to sympathicotherapy. Ed. Frison-Roche, pp.19-45.

[23]: Silano V. and Delbò M., 2008. -Assessment report on Foeniculum vulgare Miller. EMEA, EuropeanMedicines Agency. London: 23p.

[24]: Souza E.L., Stamford, T.L.M. and Lima E.O., 2006. -Sensitivity of spoiling and pathogen), 147-153.

foodrelated bacteria to Origanum vulgare L. (lamiaceae) essential oil. Brazilian Journal of Microbiology 37: pp.527-532.

[25]: TOUCHE J. (1997). -Representativity and reproducibility of plant extracts of aromatic plants, RivistaItaliana EPPOS, Special issue, p. 288-294.

[26]: Vekiari S.A., Protopapadakis E.F., Papadopoulou P, Papanicolaou D., Panou C and Vamvakias M., 2002. -Composition and seasonal variation of essential oil from leaves and peel of a lemon variety. Journal of Agrucultural and Food Chemistry 5 (1

ISSN 1737-9296