J. Mater. Environ. Sci. 7 (7) (2016) 2362-2368 *Boukeria et al.*
ISSN : 2028-2508
CODEN: JMESC

Phytochemical and physicochemical characterization of *Allium sativum* L. and *Allium cepa* L. Essential oils

S. Boukeria[*1], K. Kadi[3], R. Kalleb[1], A. Benbott[4], D. Bendjedou[2], A. Yahia[1]
[1]*Laboratory of natural sciences and materials, Mila University centre, Algeria*
[2]*Laboratoire of biology, water and the Environment Department of Biology, University 08 May, 1945 Guelma, Algeria*
[3]*Institute of Biology, University of Khenchla, Algeria*
[4]*Department of Biological Sciences, Faculty of Science, Larbi Ben M'hidi University, OEB, Algeria*

Received 09 Sep 2015, Revised 12 Feb 2016, Accepted 29 Mar 2016
**Corresponding author. E-mail: boukeriasabah@gmail.com (S. Boukeria); Phone: +213773345521*

Abstract
This study aims to investigate, the phytochemical and physicochemical characterization of the essential oils of *Allium sativum* and *Allium cepa* varieties. The varieties used for the study were graciously provided by the Technical Institute of Horticultural and Industrial Crops (ITCMI), Bir Rogaa Experimental Station, OEB (Algeria). The extraction of essential oils by steam distillation from bulbs gave high yields compared to what is reported in the literature. Red Local varieties of both species showed the highest yield with 0.72 % ± 0,015 for garlic and 0.64% ±0,032 for onion. Organoleptic and physico-chemical characteristics observed comply with AFNOR's standards. The results of phytochemical screening showed the presence of some chemical groups in one species and their absence in the other species. This was the case of the presence of saponins, alkaloids and glycosides traces in *A. sativum*, and the presence of tannins, glycosides, sterols, triterpene alkaloids and flavonoids in *A. cepa* .

Keywords: *Allium cepa* , *Allium sativum,* phytochemical screening, essential oils, physico-chemical properties

1. Introduction
Medicinal plants are sources of many active chemical compounds such as alkaloids, flavonoids, polyphenols, triterpenes, saponins, sterols, tannins, and essential oils known for their therapeutic properties. These metabolites are synthesized substances having different therapeutic properties exploited in the treatment of various diseases [1]. Plant essential oils are generally of quite complex composition containing volatile principles more or less modified during preparation [2]. They volatilize at room temperature when exposed to air [3], they are rarely colored, having a density generally lower than that of water. They have a high refractive index, are soluble in common organic solvents, in fats, in water and are easily drivable in water vapor [2].
Onion (*Allium cepa* L.) and garlic (*Allium sativum L.),* are herbaceous monocots of, the *Amaryllidaceae* family, characterized by a bulb at the base of the leaves [4]. They are among the oldest cultivated plants, used as food and in the treatment of many diseases. They have beneficial actions on cardiovascular system and on cancer [5, 6, 7]. *A. sativum* and *A. cepa* possess a large number of varieties varying in sizes, colors, flavors, and a diversified chemical composition rendering them very useful in many aspects. This research aimed to investigate the phytochemical and physicochemical characterization of the essential oils of four varieties of *A. sativum* and three varieties of *A. cepa* species.

ISSN : 2028-2508
CODEN: JMESC

2. **Experimental**

2.1. *Biological material*

Four garlic bulbs (Allium sativum L.), varieties: Messidrom, Germidour , Mocpta Bulgare and Red local and three onion bulbs (Allium cepa L.) varieties: Yellow of Spain, Red local and Red Amposta, were graciously provided by the Technical Institute of Horticultural and Industrial Crops (ITCMI), Bir Rogaa Experimental Station, Oum El Bouaghi, Algeria, (25°51' N, 5°49' E, 950 masl). This Institution had national mandate to preserve the purity all garlic and onion varieties (PGRFA: Plant Genetic Resources for Food and Agriculture 2006). The characteristics of the varieties used as plant material are given in Table 1

Table 1: Characteristics of the seven varieties tested.

Species	*Variety name*	*Origin*	*Earliness*	*Bulb*
A. sativum	Messidrome	White of Drôme	Early	Large bulb cream Caïeu
	Germidour	Purple of Cadours	Very early planting autumn	Large bulb brown Caïeu
	Mocpta Bulgare	-	Medium late	Regular bulb (13 Caïeu)
	Rouge Locale	-	Medium late	Regular bulb (14 caïeu)
A. cepa	Red Amposta	Algeria, Morocco	Early	Large bulb, purplish, transverse narrow, elliptic
	Yellow Spain	West Africa, Benin	Early	Large bulb, yellowish, transverse narrow, elliptic
	Local Red	Algeria	Early	Medium bulb, red brownish, transverse Medium, elliptic

2.2 *Phytochemicals screening*

Bulbs were used as plant material, ground with a manual grinder and weighted with an electric balance. Chemical analyses were performed in the laboratory of the Mila University Center. The different chemical groups were characterized according to procedures described in Mangambo *et al.*, [1]; Dohou *et al.*, [8]; Pange *et al.*, [9]; Trease and Evans [10] and Memelink *et al.*, [11]. The phytochemical characterization tests used are shown in table 2.

2.3. *Extraction of essential oils*

Essential oils have been obtained by the method of steam distillation using a Clevenger type distiller for 2h30 min. After extraction, essential oils are recovered, and stored, at 4°C, in sealed opaque bottles.

Yield calculation

Essential oils yield was determined according to AFNOR [12] using the following formulae

$$\mathbf{Y}(\%) = \mathbf{100}\left(\frac{MHE}{MP}\right)$$

Y: Essential oil yield, in %, MHE: Extract recovered quantity, in grams and MP: Amount of plant material subjected to extraction in grams.

2.4. Analytical study of the essential oils

The different sensory characteristics: appearance, odor, taste and color, of garlic and onion essential oils were checked and noted. Acid index, specific gravity, Miscibility in ethanol and refractive index were also determined to apprehend the quality of the extracted essential oils. These tests were applied according to procedures outlined in AFNOR [12].

Table 2: Reagents used in the identification of the various secondary metabolites.

Secondary metabolites	*Reagents*	*Indicator*
Saponosides	Foam Index > 1cm	Appearance of a persistent foam
Flavonoïdes	- Isoamyl alcohol --Hydrochloric acid (HCl) - Magnesium shavings	Orange or purplish coloration cherry red
Alcaloïdes	- Mayer & Wagner - Hydrochloric acid HCl (10%) - Ammonium hydroxide (10%) - Di ethyl ether, HCl (2%) - Alcohol 60 °	Reddish brown color Turbidity Precipitate or orange color
Tannins	- Ethanol (50%) - Ferric chloride $FeCl_3$ 2%	Black blue color
Sterols and tri-terpenes	- Acetic anhydride - Chloroform - Concentrated sulfuric acid	Purple ring turning to blue then to green
Glycosides (reducing sugars)	- Feeling Solution - Ethanol, distilled water - Sodium-potassium tartrate	Red brick color

2.4.1. Determination of physical index

- *Specific Gravity* [*AFNOR 75-111.2000*]

The density of the essential oil is the ratio of the mass of a certain volume of the essential oil and the mass of same volume of water taken at the same temperature. The measure the density of the E O was conducted by using a pycnometer and calculated from the following formulae

$$D_{20} = \frac{m2-m0}{m1-m0}$$

m_0 : The mass in grams of the empty pycnometer, m_1 : The mass in grams of the pycnometer filled with distilled water and m_2 : The mass in grams of the pycnometer filled with oil

- *Refractive index* [*AFNOR 75-112-2000*]

The refractive index is the ratio between the sine of the angle of incidence of the light beam in the air and the sine of the refraction angle of the refracted ray in the considered environment. We have conducted the measurement of refractive index of the EO using a conventional refractometer of OPTECH brand. The refractive index is given by a direct reading on the refractometer; temperature set at 20 ° C.

-*Miscibility in ethanol* [*AFNOR 75-101.2000*]

In an Erlenmeyer flask containing 1 ml of EO, through fraction of 0.2 ml and using a buret of 20 ml we pour ethanol (70%) stirring after each addition. When a limpid solution is achieved we register directly the volume of alcohol added.

2.4.2. Determination of chemical indices

- Acid index [*AFNOR –NFT-60-2000*]

The acid index (I_a) is the number of milligrams of potassium hydroxide (KOH) necessary to neutralize the free acids contained in (01) gram of the EO. The acid index is determined by the following formulae

$$I_a\ (\%) = v.c.\ \frac{56{,}11}{m}$$

V: volume in ml of the KOH solution used for the titration, C: concentration in mol / L of the KOH solution and m: mass in g of the test sample

- *Ester index* [*AFNOR NFT 75-104/1994*]

The ester index is the number of milligrams of potassium hydroxide (KOH) necessary to neutralize the liberated acid by a hydrolysis operation of the esters in basic environment.

$$I_e = \frac{28.05}{m}(V_0 - V_1)$$

V_0: Volume in ml of HCl solution (0.5N) in the blank test, V_1: Volume in ml of the HCl solution (0.5 N) measured for the calculation of I_e, m: Mass in g of the test sample and I_e: Ester index value.

- Saponification index

The saponification index is the number of milligrams of potassium hydroxide (KOH) necessary to convert into soap the fatty acids and triglycerides with an (01) gram of fats. The principle is to titrate the excess of potassium hydroxide in solution by hydrochloric acid.
Saponification index is given by the following formulae

$$I_s = \frac{N.(V_0 - V)}{W} \text{ (mg KOH/gEO)}$$

V_0: Volume in ml of the hydrochloric acid solution to the blank test, V: volume in ml of the hydrochloric acid solution used for the testing sample, N: exact normality of the hydrochloric acid solution and W: weight grams of the testing sample

- Peroxide index:

The peroxide value is the number of milliequivalents of oxygen per kilogram of fat and oxidant potassium iodide, with liberation of iodine .The principle is based on the treatment of a test sample in solution in acetic acid and chloroform, by a potassium iodide solution, then the titration of iodine with a standard solution of sodium thiosulfate.
The peroxide index is given by the following formulae

$$I_p = \frac{V_0 - V}{P} \times 10(méq.o_2\ /\ KgEO)$$

V: volume of sodium thiosulfate solution used for the test, V0: volume of sodium thiosulfate solution used for the blank test and P: portion tested (test sample) in grams

3. Results and discussion

3.1. Results

3.1.1. ***Phytochemical screening***:

Results of the phytochemical characterization of the seven varieties tested are presented in table 3.

Table 3: Results of the phytochemical analysis of garlic and onion varieties.

Metabolites	Garlic				Onion		
	Messidrom	Germidour	Mupcta Bulguar	Red local	Red local	Yellow Spain	Red Amposta
Saponosides	+	+	+	+	-	-	-
Tannins	-	-	-	-	+	±	+
Glycosides	-	-	-	+	±	±	+
Flavonoïdes	-	-	-	-	+	+	+
Alcaloïdes	+	+	+	+	+	+	+
Sterols and triterpenes	-	-	-	-	+	+	+

(+): presence, (-): absence, (±): trace.

3.1.2. *Essential oil yield*

Average oil yields, based on 1000g of fresh plant material treated were 0.46% ± 0.026, 0.72 % ± 0.015, 0.61 % ± 0.01 and 0.51% ± 0.01for Messidrom, Local Red, Germidour and Mocpta Bulguar garlic varieties, respectively ;

and 0.64% ± 0.032, 0.40% ± 0.015 and 0.52% ± 0.01 for local Red onion , Yellow Spain, Red Amposta onion varieties, respectively.

3.1.3. *Analytical study of the essential oil*
3.1.3.1 *Organoleptic characteristics of essential oils extracted*
A part from spicy flavor and liquid mobile appearance of the essential oils which are the same in both species, color is different from one variety to the other whereas odor is different from one species to another (table 4).

Table 4: Organoleptic characteristics of essential oils extracted

Varieties	*Color*	*Odor*
Garlic local Red	Yellow very pale	Strong, unpleasant ,Alliaceous
Garlic Messidrom	Yellow edge (Brownish)	Strong, unpleasant ,Alliaceous
Garlic Germidour	Yellow edge (Brownish)	Strong, unpleasant ,Alliaceous
Garlic Mocpta Bulgare	Yellow edge (dark)	Strong, unpleasant, Alliaceous
Onion Local Red	Yellow edge degraded clear	Strong,unpleasant, sulfur odor
Onion Yellow Spain	Yellow edge degraded clear	Strong, unpleasant, sulfur odor
Onion Red Amposta	Pale Yellow	Strong, unpleasant, sulfur odor

3.1.3.2 *Analytical study of the essential oil*
The main physicochemical characteristics of garlic and onion varieties essential oil are summarized in table 5. The results of the physicochemical analyzes indicated that the characteristics of essential oils were in the range of the AFNOR standards.Except Messidrom variety wich had a high acid index value compared to other varieties.

Table 5: Physical and chemical characteristics of garlic and onion varieties essential oil

	Garlic varieties				*Onion varieties*		
Parameters	LRD*	GER	MES	MOB	YSP	RAM	LRD
Acid index	6.63	6.78	10.35	7.68	5.13	4.18	8.28
Ester index	86.24	86.21	94.96	113.55	125.98	103.25	137.01
Saponification index	92.87	92.99	105.31	121.23	131.11	107.43	145.29
Peroxide index	7.97	7.13	7.13	7.15	7.12	7.11	7.14
Refractive index	1.4650	1.4640	1.4691	1.4640	1.4691	1.4651	1.4700
Specific Gravity: at 20°C	1.026	1.025	1.028	1.029	1.028	1.029	1.030
Miscibility in Ethanol 70 °	2.75	2.75	2.75	3.0	3.0	3.0	2.95

*LRD = Local red, GER = Germidor, MES = Messidrom, MOB= Mocpta Bulgar, YSP= Yellow Spain, RAM= Red Amposta, LRD= Local red.

3.2. *Discussion*
Results of phytochemical analyzes are consistent with those of Manganbu *et al.*[1] and Chitra Shenoy *et al.*[13]. The tests used show the presence of alkaloids in all varieties studied. However, tannins, flavonoids, sterols and triterpenes , are present in onion varieties and absent in garlic varieties. For saponins, the test is positive for garlic varieties. According to the results of the tests, glycosides (reducing sugars) content differs from one variety to another we notice an important presence in two varieties one for garlic (*LRD) and another for onion (RAM); however there had been a weak presence in the other two varieties of onion (LRD) and (YSP). Essential oils yield differs significantly between varieties of both species, being high in Red local variety of each species, and low in Yellow Spain onion variety and Messidrom garlic variety. Oil yields found in the present study are higher

compared to those reported in the literature. Richard [14] and Block [15] mentioned that, in onion, traces of volatile oils are found: the simple distillation of 5000g of onion is needed to obtain 233g (0.005%) of essential oil. By vacuum distillation of onion bulbs, essential oil yield found by Burdock and Fenaroli [16] and Block [15] varied from 0.005% to 0.02%, while Fabrice [17] obtained a low yield of 0.04% through simple distillation of onion bulbs. According to Amagase *et al.* [18], garlic contains approximately 0.1% to 0.3% volatile oil, in some cases up to 0.2- 0.5%, while Haciseferogullari *et al.* [19] found a yield of 0.14%. The results obtained by Emad Shalaby *et al.* [20] were as the following of (0.073% ± 0.1) for *Allium sativum* and (0.59% ± 0.0) for Allium *cepa*. We can say that variation observed in essential oils yield, between varieties of each species, may be explained by the differences in genetic background of the targeted plant and/or to the extraction protocol followed during the experiment.

Aberchane *et al.* [21] and Bourkhiss *et al.*[22] mentioned that many factors may influence yield, content, physicochemical characteristics and chemical composition of essential oils, Among these factors the species, environmental conditions, plant organ used, drying method, extraction technique, period of harvest, cultural practices and growth age of the plant material.

The results of the physicochemical tests of the essential oils are consistent with those of AFNOR standards. Specific gravity is a characteristic used in the classification of essential oils. Specific gravity of the oil is defined as the mass ratio of a volume of oil to that of an equal volume of distilled water, both held at 20 °C [12]. Measuring the Specific gravity of different essential oils showed a higher density than water allowing them to be below the water during extraction.

The acid index gives an idea about the content of free acids. Our results obtained of this index appear according to AFNOR standards, but it’s somehow higher; this can be explained by the degradation of the essential oils by hydrolysis of the esters during their storage, in contrast to an acid number less than 2 which indicates proper preservation of these oils weak quantity of free acid [23].

The refractive index depends on the chemical composition of the sample and varies essentially with the oxygenates content and monoterpenes, of which a high level of the latter gives a high index. According to Caree [24], a low refractive index of essential oils indicates low refraction of light which could promote their use in cosmetic products. Refractive index and miscibility with ethanol are in agreement with AFNOR’s standards [12].

Conclusions

The extraction results revealed a low variation of the content and the chemical composition of the essential oils of the two species tested. The phytochemical study shows, however, considerable variation among varieties of both species. This variation is explained by difference in the genetic background of the plant material tested, rather than by differences in the extraction protocol and environmental conditions which are the same. Determination of physico-chemical properties is a necessary step to identify and obtain data on the composition and purity of essential oils. This study remains unfortunately insufficient for characterization of essential oils, which need to be completed by a qualitative analysis.

Acknowledgments The authors are thankful to the research laboratory team of the Mila University Centre, Algeria, and especially Dr. Nourddine Metatla and Salha Benaissaa the teacher of English for help their help.

References

1. Mangambu M.J de Dieu., Mushagalusak F., Kadima N.J., *J. Appl. Biosci.* 75 (2014) 6211.
2. Bruneton J. Pharmacognosie, phytochimie, plantes médicinales, 3éme Ed. Tec&Doc, Lavoisier, Paris, (1999).
3. Leung A.Y. Encyclopedia of common natural ingredients used in food drugs and cosmetics, Wiley-Interscience Pub., NY, (1980).
4. Fritsch R.M., Friesen N. Evaluation, domestication and taxonomy. IN: Rabinowitch D., Currahl., Allium crop science: Recent Advance. Wallingford, CAB International, (2002) 5.
5. Virginia L., *J. chromatography A.* 1112 (2006) 3.
6. Sanjay K.B., subir K.M., *Nutr. J.,* 1 (2002) 1-14.

ISSN : 2028-2508
CODEN: JMESC

7. Sampath Kumar K.P., Debjit B., Chiranjib, Biswajit, *J. Chem. Pharm. Res.* 2 (2010) 21.
8. Dohou N., Yamni K., Tahrouch S., Idrissi Hassani L.M., Badoc A., Gmira N., *bull. Soc. Pharm. Bordeaux,* 142 (2003) 61.
9. Penge A.O., Mwelo J.N., Mbenza A.P., Tshilumbu P.K., Ngombe N.K., Kalenda N.T., Duki A.M, *Annales de Pharmacie*, 3 (2005) 153.
10. Trease E., Evans W.C. Pharmacognosy. Billiare.Tindall., London, (1987) 61.
11. Memelink J., Verpoort R., Kijne J.W. Organisation of jasmonate-responsive gene expression in alkaloid metabolism,Trends plant sci 6 (2001) 212-219.
12. AFNOR, Association Française de Normalisation, Huiles essentielles, T2, Monographie relative**s** aux huiles. 6 ème édition, Paris, (2000).
13. Chitra Shenoy, Patil M.B., Ravikumar. Swati P., *Int. J. Pharm. Pharm. Sci*. 2. Issue 2. (2009) 167-175.
14. Richard H. Les plantes aromatiques et huiles essentielles à Grasse, L'harmattan, (2005).
15. Block E. The organ sulfur chemistry of the genus *Allium*. Implication for the organic chemistry of sulphur. Angew. Chem. Int, Engl, (1992).
16. Burdock G.A., Fenaroli S., Handbook of flavor Ingredients, CRC Press Boca Raton London New York Washington, D.C. (1995).
17. Fabrice B., Les huiles essentielles-Découvrir les bienfaits et les vertus d'une médecine ancestrale; Fernand Lanore ; ISBN : 978-2-85157-566
18. Amagase H., Petesch B.L., Matsuura H., Kasuga S., Itakura Y., *J. Nutr.* 131 (2001) 955s.
19. Haciseferogullari H., Ozcan M., Demir F., Calisir S., *J. Food Eng.* 68 (2005) 463.
20. Emad A. Shalaby, Nasr N.F., Samia M. El Sherief., *J. Medicinal Plants Res.* 5 (2011) 922.
21. Aberchane M., Fechtal M., Chaouche A., Bouayoune T., *Annales de la recherche forestière au Maroc* 34 (2001) 110.
22. Bourkhiss M., Hnach M., Lakhilifi T., Boughdad A., Farah A., Satrani B., *Les Technologies de Laboratoire,* 6 (2011) 64.
23. Kanko C., Sawaliho B.E., Kone S., Koukoua G., N'guessant Y.T., *Comptes rendus Chimie* 7 (2004) 1039.
24. Caree P. Précis de technologie et de chimie industrielle. T3, Balliére JB. et fils, (1953).

(2016) ; http://www.jmaterenvironsci.com