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

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Received 09 Sep 2015, Revised 12 Feb 2016, Accepted 29 Mar 2016
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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.
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.

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

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

\[ Y(\%) = 100 \left( \frac{MHE}{MP} \right) \]

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>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>Reagents</th>
<th>Indicator</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saponosides</strong></td>
<td>Foam Index &gt; 1cm</td>
<td>Appearance of a persistent foam</td>
</tr>
<tr>
<td><strong>Flavonoïdes</strong></td>
<td>Isoamyl alcohol, Hydrochloric acid (HCl), Magnesium shavings</td>
<td>Orange or purplish coloration, cherry red</td>
</tr>
<tr>
<td><strong>Alcaloïdes</strong></td>
<td>Mayer &amp; Wagner, Hydrochloric acid (10%), Ammonium hydroxide (10%), Diethyl ether, HCl (2%), Alcohol 60 °</td>
<td>Reddish brown color, Turbidity, Precipitate or orange color</td>
</tr>
<tr>
<td><strong>Tannins</strong></td>
<td>Ethanol (50%), Ferric chloride FeCl₃ 2%</td>
<td>Black blue color</td>
</tr>
<tr>
<td><strong>Sterols and tri-terpenes</strong></td>
<td>Acetic anhydride, Chloroform, Concentrated sulfuric acid</td>
<td>Purple ring turning to blue then to green</td>
</tr>
<tr>
<td><strong>Glycosides (reducing sugars)</strong></td>
<td>Feeling Solution, Ethanol, distilled water, Sodium-potassium tartrate</td>
<td>Red brick color</td>
</tr>
</tbody>
</table>

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 EO was conducted by using a pycnometer and calculated from the following formulæ

\[
D_{20} = \frac{m_2 - m_0}{m_1 - m_0}
\]

- **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 (Iₐ) 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 formulæ

\[
I_a \ (\%) = \frac{V \times C}{56.11 \ \text{m}}
\]

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

\[ I_p = \frac{V_0 - V}{P} \times 10(m \cdot eq. O_2/Kg \cdot EO) \]

\( V \): volume of sodium thiosulfate solution used for the test, \( V_0 \): 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.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Garlic</th>
<th>Onion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Messidrom</td>
<td>Germidour</td>
</tr>
<tr>
<td>Saponosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alcaloïdes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sterols and triterpenes</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) : 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% ± 0.01 for 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

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Color</th>
<th>Odor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Garlic local Red</strong></td>
<td>Yellow very pale</td>
<td>Strong, unpleasant, Alliaceous</td>
</tr>
<tr>
<td><strong>Garlic Messidor</strong></td>
<td>Yellow edge (Brownish)</td>
<td>Strong, unpleasant, Alliaceous</td>
</tr>
<tr>
<td><strong>Garlic Germidour</strong></td>
<td>Yellow edge (Brownish)</td>
<td>Strong, unpleasant, Alliaceous</td>
</tr>
<tr>
<td><strong>Garlic Mocpta Bulgare</strong></td>
<td>Yellow edge (dark)</td>
<td>Strong, unpleasant, Alliaceous</td>
</tr>
<tr>
<td><strong>Onion Local Red</strong></td>
<td>Yellow edge degraded clear</td>
<td>Strong, unpleasant, sulfur odor</td>
</tr>
<tr>
<td><strong>Onion Yellow Spain</strong></td>
<td>Yellow edge degraded clear</td>
<td>Strong, unpleasant, sulfur odor</td>
</tr>
<tr>
<td><strong>Onion Red Amposta</strong></td>
<td>Pale Yellow</td>
<td>Strong, unpleasant, sulfur odor</td>
</tr>
</tbody>
</table>

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 which had a high acid index value compared to other varieties.

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Garlic varieties</th>
<th>Onion varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid index</td>
<td>LRD*</td>
<td>GER</td>
</tr>
<tr>
<td>Ester index</td>
<td>86.24</td>
<td>86.21</td>
</tr>
<tr>
<td>Saponification index</td>
<td>92.87</td>
<td>92.99</td>
</tr>
<tr>
<td>Peroxide index</td>
<td>7.97</td>
<td>7.13</td>
</tr>
<tr>
<td>Refractive index</td>
<td>1.4650</td>
<td>1.4640</td>
</tr>
<tr>
<td>Specific Gravity: at 20°C</td>
<td>1.026</td>
<td>1.025</td>
</tr>
<tr>
<td>Miscibility in Ethanol 70 °</td>
<td>2.75</td>
<td>2.75</td>
</tr>
</tbody>
</table>

*LRD = Local red, GER = Germidor, MES = Messidrom, MOB = Mocpta Bulgare, 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. Mourad Metatla and Sáha Benaïssa for their help.

References

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