Drying effect on yield and antimicrobial activity of essential oils

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Abstract: The present paper highlights the drying effect on yield and activity of some medicinal and aromatic plants. For this purpose we have chosen nine plants, four trees and five herbs. Each plant species represents one of the great aromatic families in Mediterranean. Drying was carried out at the dark at room temperature. Essential oils were obtained by hydrodistillation, where, we have noticed a significant decrease in yield of essential oil after drying in all plants. This loss was in strong relation with moisture decrease by drying. Drying effect on antimicrobial activity of essential oils has been evaluated by agar disk diffusion method. We have found a significant decrease in antimicrobial activity among all active essential oils after drying. Finally, we have concluded that drying significantly affects the essential oil quantity and quality.

Keywords: Antimicrobial activity; drying; essential oils; yield.

Introduction

Many herbalists are interested in the need to dry the plant material for several reasons including the exclusion of moisture (Müller and Heindl 2006) and the facilitation of the extraction process (Marur and Sodek 1995) for various phytochemicals to obtain their highest yield (Birdi et al. 2006). The storage of plants for use in a later time (Singh 2009) or for trade purpose requires drying (Douglas et al. 2005; Jakhar et al. 2009). According to some authors, the fresh plant material should be delivered as quickly as possible to the processing facility to prevent microbial fermentation or thermal degradation by mentioning that the methods and temperatures used for drying may have a considerable impact on the quality of the resulting medicinal plant materials (Birdi et al. 2006), while others use the fresh plant material without justifying the reason for this type of use (Lucchesi et al. 2004; Okoh et al. 2010; Stashenko et al. 2004). In contrast, some authors confirm that drying reduces the yield of volatile oil while the extraction from fresh plants is economic which avoids the drying process (Schmidt 2010).

Essential oils extracted from many medicinal plants have several interesting biological activities (Reichling et al. 2009). The antimicrobial activity is strongly correlated with the content of terpenoid phenols such as carvacrol, eugenol and thymol (Edris 2007; Guarda et al. 2011) and some other oxygenated monoterpenes such as nerol, linalool, α-terpineol, fenchol and terpinen-4-ol (Kotan et al. 2007). From the phenolic compounds, eugenol and thymol reveal a broadband spectrum of activity in various in vitro test systems due to their considerable water solubility and volatility (Pauli and Schilcher 2010). However, those molecules are characterized by low molecular weight (Bakkali et al. 2008).

The sensitivity of volatile constituents from various aromatic plants is observed in the changes of their concentrations during drying which depends on several factors such as drying method and parameters that are characteristic of the product subjected to drying (Venskutonis 1997). Even the freeze-drying, one of the best...
pharmaceutical techniques for drying medicinal plants, causes a change in the relative concentrations of molecules in the essential oil (Abascal et al. 2005) which may influence the pharmacological properties of these medicinal plants. This drying method causes the loss of certain monoterpenes such as 1,8-cineole, linalool and geraniol (Díaz-Maroto et al. 2002). Same case is observed in natural drying and in hot air drying at 45 °C (Omidbaigi et al. 2004; Szumny et al. 2010). In general, convective drying (using hot air) leads to significant volatile losses (Díaz-Maroto et al. 2004), with losses increasing with higher air temperatures and longer drying times. However, the loss of volatiles was maximum in the first step of the drying process and insignificant thereafter (Szumny et al. 2010). Therefore, shade drying is the preferred method for drying plant material since it can maintain or minimize loss of color of leaves and flowers (Birdi et al. 2006).

The antimicrobial activity is one of the most important therapeutic effects that the plant may possess (Cowan 1999; Pauli and Schilcher 2010). The change in the composition of extracts has a significant impact towards these microorganisms whether in the emergence of their sensitivity or resistance (Bitu et al. 2012; Mondal et al. 2007; Sasidharan and Menon 2010). Hence, it may appear evident the importance for the plant to keep most of its components, especially quickly volatile ones, which is somewhat guaranteed in the fresh case (Al-Jaber et al. 2012; Baritaux et al. 1992; Englund and Nussbaum Ralph 2000; Jerković et al. 2001).

In the absence of a large comparative antimicrobial analysis between extraction in the case of dry and fresh plant material, which is seen in most of what is published. So, we decided to perform a simple work to compare between the amount and effect of essential oils for a group of plants known by their medical properties in both dry and fresh cases.

**Materials and methods**

**Plant material**

We have selected a group consisting of nine species; each one represents a certain family for several reasons. First, there are differences in type secretory containers and biosyntheses of essential oil taxonomical families (Franz and Novak 2010). While, this distinctive feature between botanical families may affect the way these plants wither, and more specifically, changes that may affect the chemical composition of the essential oil during drying. Secondly, selected species are the most conserved plants in our regions for subsequent uses either medical or food.

Apium graveolens L. (Apiaceae), Citrus × sinensis (L.) Osbeck (Rutaceae), Cupressus sempervirens L. (Cupressaceae), Dittrichia viscosa (L.) Greuter subsp. viscosa (Asteraceae), Eucalyptus globulus Labill. subsp. globules (Myrtaceae), Laurus nobilis L. (Lauraceae), Lavandula multifida L. (Lamiaceae), Pelargonium graveolens L’Hér. (Geraniaceae) and Pistacia lentiscus L. (Anacardiaceae) plant material have been harvested from the region of Tlemcen located in the northwestern Algeria from November 2011 to January 2012, from which we find those in the wild and others cultivated. We have used organs of their aerial part for extraction which are summarized in (Table 1). Plant material was divided into two parts by weighing each of them separately, a part for extraction in the fresh case which is launched the day its harvest, and the other part which is left to dry for a period ranging from 20 to 30 days by spreading it in open air and away from sunlight. Specimens of all spices in this study were identified by Laboratory of Ecological Management of Natural Ecosystems; University of Tlemcen. All voucher specimens were deposited at LAMAABE laboratory, University of Tlemcen.

**Obtaining the essential oils**

For this purpose, we have used hydrodistillation, where obtaining the essential oil was performed in a Clevenger-type apparatus, by putting plant material in direct contact with water inside a flask over a heat source. The flask was connected to a column then a condenser allowing the accumulation of
water vapor loaded with essential oil droplets. Essential oil was collected in a graduated burette (precise volume of 0.05 ml) where the volume is read directly. The essential oil was dried using magnesium sulfate and stored in smoked vials at +4 °C.

Table 1: Data on the studied plant material.

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Family</th>
<th>Studied Organs</th>
<th>Harvest Station</th>
<th>Location</th>
<th>Altitude (m)</th>
<th>Total Annual QPF*</th>
<th>Harvest Date</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Apium graveolens</em> L.</td>
<td>Apiaceae</td>
<td>Leaves &amp; Stems</td>
<td>Ain El-Houtz (Chetouane)</td>
<td>+34°56'14&quot;</td>
<td>476 m</td>
<td>450 mm</td>
<td>Jan-12</td>
</tr>
<tr>
<td><em>Citrus sinensis</em> (L.) Osbeck</td>
<td>Rutaceae</td>
<td>Fruit Cortex</td>
<td>DjenaneLihoudi (Hennaya)</td>
<td>+34°57'48&quot;</td>
<td>379 m</td>
<td>450 mm</td>
<td>Nov-11</td>
</tr>
<tr>
<td><em>Cupressus sempervirens</em> L.</td>
<td>Cupressaceae</td>
<td>Leaves &amp; Fruits</td>
<td>Bouhannak (Mansourah)</td>
<td>+34°52'37&quot;</td>
<td>749 m</td>
<td>450 mm</td>
<td>Dec-11</td>
</tr>
<tr>
<td><em>Dittrichia viscosa</em> (L.)</td>
<td>Asteraceae</td>
<td>Leaves &amp; Stems</td>
<td>Imama (Mansourah)</td>
<td>+1°21'10&quot;</td>
<td>716 m</td>
<td>450 mm</td>
<td>Dec-11</td>
</tr>
<tr>
<td><em>Eucalyptus globulus</em></td>
<td>Myrtaceae</td>
<td>Leaves</td>
<td>Bouhannak (Mansourah)</td>
<td>+34°52'37&quot;</td>
<td>749 m</td>
<td>450 mm</td>
<td>Nov-11</td>
</tr>
<tr>
<td><em>Labill. subsp. globulus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Laurus nobilis</em> L.</td>
<td>Lauraceae</td>
<td>Leaves</td>
<td>Bouhannak (Mansourah)</td>
<td>+34°53'3&quot;</td>
<td>701 m</td>
<td>450 mm</td>
<td>Nov-11</td>
</tr>
<tr>
<td><em>Lavandula multifida</em> L.</td>
<td>Lamiacae</td>
<td>Leaves, Stems &amp; Flowers</td>
<td>OuledCharef (Maghnia)</td>
<td>+34°53'2&quot;</td>
<td>697 m</td>
<td>450 mm</td>
<td>Jan-12</td>
</tr>
<tr>
<td><em>Pelargonium graveolens</em> L’Hér.</td>
<td>Geraniaceae</td>
<td>Leaves &amp; Stems</td>
<td>Imama (Mansourah)</td>
<td>+34°53'35&quot;</td>
<td>715 m</td>
<td>450 mm</td>
<td>Jan-12</td>
</tr>
<tr>
<td><em>Pistacia lentiscus</em> L.</td>
<td>Anacardiaceae</td>
<td>Leaves &amp; Fruits</td>
<td>Bouhannak (Mansourah)</td>
<td>+34°53'1&quot;</td>
<td>690 m</td>
<td>450 mm</td>
<td>Jan-12</td>
</tr>
</tbody>
</table>

* 2011 total annual quantitative precipitation forecast according to Office National de Meteorologie d’Algerie in March 2012 (http://www.meteo.dz/).

Strains and media

Seven strains have been selected, one of them is a fungus and the rest are bacteria representing various infection sources. The fungal strain was yeast which is *Candida albicans* ATCC 10231. While we find among the bacterial strains, three Gram-negative ones which are *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 70603, and three Gram-positive ones which are *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633.

The purity of strains was verified by subculturing them in their selective growth media and taking distinct colonies in each time, where *Candida albicans* was cultivated in Sabouraud agar (Fluka®, India) at 37 °C for 24 h, *Enterococcus faecalis* in Bile Esculin agar (Fluka®, Switzerland) at 37 °C for 24 h, *Staphylococcus aureus* in Mannitol Salt agar (Fluka®, Switzerland) at 37 °C for 24 h, and *Bacillus subtilis* in Mossel agar (Fluka®, Switzerland) at 30 °C for 24 h. As media for testing antimicrobial effect, we have chosen Mueller-Hinton agar (Fluka®, India) for bacteria and Sabouraud agar (Fluka®, India) for yeasts.

Microbial suspensions were prepared in test tubes containing 5 ml of Mueller-Hinton broth (Fluka®, India) for bacteria and Sabouraud broth (Fluka®, India) for yeast, as blank.

Previously purified colonies were taken and inoculated into tubes with 5 ml of Mueller-Hinton broth (Fluka®, India) for bacteria and Sabouraud broth (Fluka®, India) for yeast, at 37 °C for 24 h. After incubation, suspensions were shaken well using the vortex then diluted for standardizing, so that the inoculum was set to 0.5 McFarland which corresponds to an optical density from 0.08 to 0.1 at 625 nm wavelength.
The inoculum final concentration will be approximately $10^8$ cfu/ml.

**Evaluation of antimicrobial effect**

We have used Kirby-Bauer’s agar disk diffusion method (Bauer et al. 1966), where filter paper disks impregnated with 2 µl of essential oil are placed on the surface of agar pre-inoculated by swabbing of standardized microbial suspension, each disk has a 6 mm diameter. After incubation, the results are read by measuring the diameter of inhibition zones in millimeters (mm) by Vernier scale. Gentamicin (10 µg per disc) and Amphotericin B (100 µg per disc) (Oxoid, England) were used and served as positive controls for bacterial and fungal strains respectively. All tests were performed in triplicate.

**Statistical analysis**

The effect of plant weight decrease on essential oil yield was verified by a correlation test ($P \leq 0.05$). The difference in antimicrobial activity of essential oils extracted in both fresh and dry cases was verified by two-way ANOVA test ($P \leq 0.05$) for each strain. All tests have been carried out using Microsoft® Excel.

**Results**

The results of the extraction yields are presented in (Table 2). Where, we noticed a significant decrease in yield of essential oils after drying in all plants in this study. These losses of yield are remarkably large in some species such as *Citrus sinensis* and *Apium graveolens*, while this change is less significant in other one such as *Laurus nobilis*. Moreover, there was an almost total loss of essential oil after drying observed in a species of the Asteraceae family, *Dittrichia viscosa*.

**Table 2:** Essential oils yield difference between fresh and dry cases.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Fresh weight (g)</th>
<th>Weight after drying (g)</th>
<th>Weight decrease (%)</th>
<th>Fresh yield (%)*</th>
<th>Yield after drying (%)*</th>
<th>Yield decrease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Apium graveolens</em></td>
<td>1000</td>
<td>217</td>
<td>78</td>
<td>0.06</td>
<td>0.03</td>
<td>50</td>
</tr>
<tr>
<td><em>Citrus sinensis</em></td>
<td>1000</td>
<td>167</td>
<td>83</td>
<td>0.45</td>
<td>0.14</td>
<td>68</td>
</tr>
<tr>
<td><em>Cupressus sempervirens</em></td>
<td>1000</td>
<td>495</td>
<td>51</td>
<td>0.16</td>
<td>0.13</td>
<td>21</td>
</tr>
<tr>
<td><em>Dittrichia viscosa subsp. viscosa</em></td>
<td>1000</td>
<td>241</td>
<td>76</td>
<td>0.10</td>
<td>0.01</td>
<td>98</td>
</tr>
<tr>
<td><em>Eucalyptus globulus subsp. globulus</em></td>
<td>1000</td>
<td>423</td>
<td>58</td>
<td>0.70</td>
<td>0.58</td>
<td>18</td>
</tr>
<tr>
<td><em>Laurus nobilis</em></td>
<td>1000</td>
<td>500</td>
<td>49</td>
<td>0.62</td>
<td>0.59</td>
<td>05</td>
</tr>
<tr>
<td><em>Lavandula multifida</em></td>
<td>1000</td>
<td>423</td>
<td>58</td>
<td>0.10</td>
<td>0.08</td>
<td>20</td>
</tr>
<tr>
<td><em>Pelargonium graveolens</em></td>
<td>1000</td>
<td>318</td>
<td>68</td>
<td>0.13</td>
<td>0.08</td>
<td>41</td>
</tr>
<tr>
<td><em>Pistacia lentiscus</em></td>
<td>1000</td>
<td>494</td>
<td>51</td>
<td>0.12</td>
<td>0.11</td>
<td>08</td>
</tr>
</tbody>
</table>

*Yield percentage was determined by volume/weight ratio.

On the other hand, we have observed that loss of essential oil yield in plants after drying was related to the decrease of their weight. Where, species that lose a lot of weight by drying, their yield decreases sharply and vice versa. Furthermore, we have clearly observed highly significant correlation (R=0.86) between plant material weight decrease and essential oil yield decrease after drying (Figure 1).

The antimicrobial activity of studied plants was summarized in (Table 3). Where, we can see that *Lavandula multifida* has the most clear inhibitory effect on most strains especially on Gram-positive bacteria. Then, *Pelargonium graveolens* and *Eucalyptus globulus* have a good inhibitory effect on each of *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans*. It has also been shown that *Dittrichia viscosa*, *Pistacia lentiscus* and *Apium graveolens* have an acceptable inhibitory effect on each of *Candida albicans*, *Bacillus subtilis* and *Staphylococcus aureus*, respectively. While, *Cupressus sempervirens*, *Citrus sinensis* and *Laurus nobilis* haven’t shown any inhibitory effect that can be mentioned.
Drying effect on essential oils of some MAP's

Figure 1: Correlation between plant material weight decrease and essential oil yield decrease.

Table 3: Inhibition zones diameters.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Essential oils (2 µl)</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>F</td>
<td>27 ± 1</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>14 ± 0</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>F</td>
<td>20 ± 1</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>F</td>
<td>16 ± 1</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>F</td>
<td>09 ± 1</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>F</td>
<td>14 ± 1</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>F</td>
<td>06 ± 0</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>F</td>
<td>26 ± 0</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>14 ± 1</td>
</tr>
</tbody>
</table>

Abbreviation: Mean ± Standard Deviation (SD) of three repeats, 6.0 = no inhibition zone.

We can even see clearly the retraction of the inhibitory effect of all active essential oils after drying, especially for *Lavandula multifida*, and with less degree, *Pelargonium graveolens*, *Eucalyptus globulus*, *Dittrichia viscosa*, *Pistacia lentiscus* and *Apium graveolens*. It should be denoted the lack of sensitivity for both *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* to all essential oils except the low inhibitory effect observed with those of *Lavandula multifida* and *Pistacia lentiscus* on *Klebsiella pneumoniae* and that of *Apium graveolens* on *Pseudomonas aeruginosa* only in the fresh case.

**Discussion**

**Essential oils yields**

Extraction results show a clear difference in yield which was turned down after drying in all studied plants. This confirms many results in studies carried out in this area by mentioning the evident effect of drying (Jerković et al. 2001; Okoh et al. 2008) whether on yield decrease or on the change of essential oil chemical composition by concentration decrease of some constituents (Al-Jaber et al. 2012; Baritaux et al. 1992; Díaz-Maroto et al. 2004; Englund and Nussbaum Ralph 2000; Figiel et al. 2010; Jerković et al. 2001; Okoh et al. 2008). While, other authors have stated increase in yield of essential oil after drying with reference to dry weight. In fact, there was a decrease towards fresh weight (Combrinck et al. 2006).

On the other hand, the decrease in weight observed after drying is expressed by the decrease in moisture to preserve the product for extended shelf life (Müller and Heindl 2006). Thus, the correlation observed between material weight decrease and essential oil yield decrease after drying is in fact a relationship between yield loss and moisture decrease during drying. While, plants drying contributes significantly to the loss of moisture which is expressed by the content of water and all of what is volatile among other fluids (Isengard 1995). So, essential oil constitutes one of the most important moisture components in plant especially its highly volatile constituents. From here, we can say that drying has a clear effect on essential oil yield decrease, so it seems very important to perform extraction in the fresh case for several reasons particularly to maintain maximum amount of these essential oils.

Drying may have a role in total or partial loss of essential oil which can be seen in *Dittrichia viscosa* by the fact that it’s essential oil is characterized by high viscosity and quickly volatile constituents may occur in small amounts with regard to the other ones having a high molecular weight which gives the viscous property for the essential oil (Stewart 2005). The possibility to obtain a viscous essential oil rich in high molecular weight constituents in a short time may explain their extraction easiness and finally their loss possibility after drying, so that what have been seen in fact in this study.

**Essential oils effect on studied strains**

The results of the antimicrobial activity of essential oils have shown a clear influence of drying on the antimicrobial activity of these oils, which was turned down after drying in most active plants. Anyway, a few studies have reported the comparison of essential oil antimicrobial activity between fresh and dry cases. The decrease of antibacterial activity was clearly observed after drying for *Ocimum sanctum* (Mondal et al. 2007), *Zingiber officinale* (Sasidharan and Menon 2010) and *Lippia gracilis* (Bitu et al. 2012). While, there was an increase in antifungal activity after drying for *Ocimum sanctum* essential oil (Mondal et al. 2007).

The significant increase in activity observed with essential oils extracted from fresh plants have been reported by some authors due to quantitative and qualitative variations in the essential oil chemical composition (Mondal et al. 2007; Sasidharan and Menon 2010). Thus, drying effect on chemical composition has been well discussed in many studies. For example, there was a decrease in monoterpene concentration after drying while sesquiterpenes were in considerable amounts in comparison with the fresh case (Al-Jaber et al. 2012; Bartley and Jacobs 2000; Díaz-Maroto et al. 2002; Sasidharan and Menon 2010; Szumny et al. 2010). Furthermore, the decrease in
monoterpenes is due to their low weight and high volatility compared to other compounds in essential oil (Bartley and Jacobs 2000; Stewart 2005). Essential oil antimicrobial activity is highly correlated with the composition in monoterpenes compared with other chemical families and in oxygenated molecules against hydrocarbons (Sinha and Gulati 1990; Srivastava et al. 2000). Then, monoterpenes-sesquiterpene ratio specifies the essential oil quality (Reverchon 1997). So, we can say that the retraction of the antimicrobial activity seen in our results is probably reported to the decrease in concentration of monoterpenes lost by drying, especially oxygenated ones.

Conclusion

Drying of aromatic plants affects significantly the quantity and the quality of essential oils. Therefore, the extraction from fresh plants not only economically increases the yield for industries, but also the pharmaceutical quality of essential oils.

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