The effects of hydrodistillation and solvent free microwave extraction methods on the chemical composition and toxicity of essential oils from the leaves of *Mentha longifolia* L. subsp. *capensis*.

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Accepted 23 November, 2011

The effects of hydrodistillation (HD) and solvent free microwave extraction (SFME) methods on the chemical composition and toxicity of *Mentha longifolia* subsp. *capensis* leaves were studied. Colourless oils of 1.52 and 1.63% (wet/dry weight) were obtained from HD and SFME methods, respectively. Analyses of the two oils revealed a total of 21 components. These accounted for about 80% of the total oil composition. The HD oil was found to be characterized by monoterpenoids with oxygenated monoterpenes, such as menthone (41.9%), α-pinene (5.4%), pulegone (34.2%), β-pinene (2.4%) and 1, 8-cineole (6.5%). Other identified constituents, include cis-sabinene hydrate (0.4%), linalool (1.0%), piperitenone (1.3%), β-caryophyllene (0.4%), germacrene D (0.5%) and isomenthone (0.4%). However, the hydrodistilled oil showed the presence of 3 octanol (0.2%), bicyclogermacrene (0.1%) and caryophyllene oxide (0.1%) which were not found in the oil extracted with solvent free microwave extractor. The SFME oil was characterized by menthone (28.0%), α-pinene (3.6%), pulegone (23.0%), β-pinene (1.0%) and 1, 8-cineole (5.4%). Other identified constituents, include cis-sabinene hydrate (0.1%), linalool (0.5%), piperitenone (0.1%), β-caryophyllene (0.3%), germacrene D (0.2%) and isomenthone (0.3%). In vitro cytotoxicity test was also studied by brine shrimp lethality bioassay. At 40 µg/ml which was the least concentration used, the oil was not toxic; all the brine shrimps survived. While maximum mortalities happened at 200 µg/ml, the least mortalities were observed at 40 µg/ml.

Key words: *Mentha longifolia*, essential oils, hydrodistillation, solvent free microwave extraction, toxicity.

INTRODUCTION

*Mentha longifolia* L., also known as “wild mint” is widely distributed throughout Southern Africa as well as in some parts of Botswana, Namibia and Zimbabwe. The plant is a perennial herb common in wet places, with creeping rhizomes and erect flowering stems of up to 80 cm in height. The aerial part of the plant are highly aromatic with a typical mint smell (Codd and Leistner, 1985). The aerial part is commonly used in folk medicine for the treatment of cold, cough, asthma, chest inflammation, including pulmonary tuberculosis, fever, indigestion and gynaecological ailments. It is also used externally to treat wounds and swollen glands (Van wyk et al., 1997; Batten et al., 2001; Hutchings, 1996).

The main component of the essential oil of *M. longifolia* from South Africa are monoterpenene ketone and menthone (Oyedeji and Afolayan, 2006). This is a different chemotype as compared to those in other parts of the world, which have carvone (Younis and Beshir, 2004), piperitone (Karousou et al., 1998) and piperitenone, and its oxide (Venskutonis, 1996) as the major constituents. These menthane monoterpen oxide give *M. longifolia* as its characteristic odour. Some other significant constituents present in almost all the chemotypes are β-caryophyllene, germacrene D, limonene, pulegone, β-pinene and 1, 8-cineole.

The oils of *Mentha* species are known to contain
hesperidin and several other flavonoids (Bourweig and Pohl, 1973) as well as numerous monoterpenoids with menthol, carvone, limonene and menthone as dominating compounds (Bruneton, 1995). However, there have been reports of some variations in the oil constituents from different countries (Van Wyk et al., 1997). Essential oil from *M. longifolia* has been reported to possess antioxidant and antimicrobial properties (Oyedeji and Afolayan, 2006; Kaur and Kapoor, 2002; Daferera et al., 2003; Gulluce et al., 2007).

Essential oils are volatile, natural and complex compounds characterized by strong odor and are produced by aromatic plants as secondary metabolites. They are very complex natural mixtures which can contain 20 to 60 components at quite different concentrations (Bakkalli et al., 2008). Essential oils are usually isolated by traditional hydrodistillation (HD), steam distillation or organic solvent extraction methods. Unfortunately, losses and degradation of some volatile compounds due to long extraction times, degradation of unsaturated or ester compounds through thermal or hydrolytic effects are the principal disadvantages of these extraction methods (Khajeh et al., 2004; Tuan and Lliangantieke, 1997). For example, monoterpenes are well known to be vulnerable to chemical changes under steam distillation conditions and even conventional solvent extraction is likely to involve losses of more volatile compounds during the removal of the solvent (Presti et al., 2005).

Recently, the supercritical fluid extraction of rosemary with CO₂ has been a subject of research (Carvalho et al., 2005) and has become a valid alternative to the conventional extraction procedures mainly, because the dissolving power of the extracting medium can be adjusted by regulating the pressure and temperature conditions. Today, an alternative method for extracting natural products by using microwave energy has been developed (Lucchesi et al., 2003). Solvent free microwave extraction (SFME) is based on the combination of how microwave heating and distillation is performed at atmospheric pressure. The SFME appeared to be particularly attractive for the isolation of essential oil from rosemary (Chen and Spiro, 1995).

Some of the advantages of this method over hydrodistillation (HD) includes, rapidity in attaining the extraction temperature of 100°C for the first essential oil droplet, high yield of essential oil, lower energy requirement and high purity of the oil extracted (Lucchessi et al., 2004). According to Smith et al. (2005), in order to obtain essential oils of constant composition, they have to be extracted under the same conditions from the same organ of plant which has been growing on the same soil, under the same climate and harvested in the same season.

Although, several workers have reported variations in chemical compositions of essential oils due to their origin, environmental condition and the developmental stage of collected plant materials, no such information is available on the influence of extraction methods on the chemical composition of essential oil of *M. longifolia*.

In the present study, the effects of different extraction methods on the chemical compositions of the oils from the leaves of *M. longifolia* were investigated. We also examined the toxicity of the oils on brine shrimps. The brine shrimp lethality assay is considered a useful and simple tool for preliminary assessment of toxicity (Dosumu et al., 2010).

**MATERIALS AND METHODS**

**Collection of plant materials and extraction of the essential oil**

The processing of the plant materials and extraction of the essential oils using HD and SFME methods were in accordance with our previous report (Okoh et al., 2010). Briefly, two samples of fresh leaves (250 g each) of *M. longifolia* were collected in May, 2011 from Nkonkobe Municipality in the Eastern Cape province of South Africa (latitudes 30° 00 to 34° 15S and longitudes 22° 45 to 30° 15E). The plant was identified by Professor Don Grierson of the Department of Botany, University of Fort Hare, Alice, and a voucher specimen (Okoh/12) was deposited at the University herbarium.

SFME was carried out with a Milestone DryDIST (2004) apparatus. The multimode reactor has a twin magnetron (2 x 800 W, 2450 MHz) with a maximum delivered power of 500 W in 5 W increments. A rotating microwave diffuser ensures homogeneous microwave distribution throughout the plasma coated PTFE cavity. The temperature was monitored by an external infrared (IR) sensor. Constant conditions of temperature and water were guaranteed by the reflux of condensed water, which was achieved by a circulating cooling system at 5°C. 250 g of the leaves were placed into the reactor without addition of water or any solvent. The exhaustive extraction of the essential oil was obtained in 40 min.

For the HD, 250 g of the plant leaves were hydrodistilled for 3 h in an all-glass clevenger apparatus in accordance with British Pharmacopoeia (1980). Heat was supplied to the heating mantle (50°C) and the essential oil was extracted with 4 L of water for 3 h (until no more essential oil was coming out). The oils collected from both extraction methods were analyzed immediately after each collection using gas chromatography-mass spectrometry (GC-MS).

**Gas chromatography mass spectrometry (GC/MS)**

GC-MS analyses were performed on a Hewlett-Packard HP 5973 mass spectrometer interfaced with an HP-6890 gas chromatograph. The following column and temperature conditions were used: initial temperature 70°C, maximum temperature 325°C, equilbria tion time 3 min, ramp 4°C min⁻¹, final temperature 240°C; inlet: split less, initial temperature 220°C, pressure 8.27 psi, purge flow 30 ml/min, purge time 0.20 min, gas type helium; column: capillary, 30 m x 0.25 mm i.d., film thickness 0.25 µm, initial flow 0.7 ml/min, average velocity 32 cm/s; MS: EI method at 70 eV.

**Identification of components**

The individual constituents of each oil were identified on the basis of their retention indices determined with a reference to a homologous series of n-alkanes and by comparison of their mass spectral fragmentation patterns with data previously reported in literature (McLafferty and stauffer, 1989; Adams, 2001; Joulain and Konig, 1998). The yield of each component was calculated per kg
of the plant material, while its percentage composition was calculated from summation of the peak areas of the total oil composition.

**Brine shrimp lethality test**

The brine shrimp lethality test was used to predict the toxicity of the oils and was conducted according to the methods of Meyer et al. (1982), Falope et al. (1993) and Oloyede et al. (2010) using brine shrimp eggs obtained from Ocean Star International, Inc. Company USA.

The shrimp eggs were hatched in sea water for 48 h at room temperature. The naupili (harvested shrimps) were attracted to one side of the vials with a light source. Solutions of the oils were made in dimethyl sulfoxide (DMSO), at varying concentrations (200, 160, 120, 80 and 40 µg/ml) and incubated in triplicate vials with the brine shrimp larvae. Ten brine shrimp larvae were placed in a mixture of sea water and DMSO only as control. After 24 h, the vials were examined against a lighted background and the average number of larvae that survived in each vial was counted.

**RESULTS AND DISCUSSION**

Colourless oils of 1.52 and 1.63 % (weight/dry weight) were obtained from the leaves of *M. longifolia* through hydrodistillation and solvent free microwave extraction methods, respectively. Analyses of the oils together revealed overall total of 64.6% for solvent free microwave extraction and 96.5% for hydrodistilled oil. The two oils were characterized by monoterpenoids with oxygenated monoterpenes dominating their compositions. However, the hydrodistilled oil showed the presence of 3-octanol (0.2%), bicyclogermacrene (0.1%) and caryophyllene oxide (0.1%) in small quantities which were only present in trace amounts in the oil extracted with solvent free microwave extractor.

Major compounds were menthone (HD = 41.9%; SFME = 28%), α-pinene (HD = 5.4%; SFME = 3.6%), pulegone (HD = 34.2%; SFME = 23%), β-pinene (HD = 2.4%; SFME = 2.0%) and 1,8-cineole (HD = 6.5%; SFME = 5.4%). Other identified constituents include cis-sabinene hydrate (HD = 0.4%; SFME = 0.1%), linalool (HD = 1.0%; SFME = 0.5%) piperitenone (HD =1.3% ; SFME = 0.1%), β-caryophyllene (HD = 0.4%; SFME = 0.3%), germacrene D (HD = 0.5%; SFME = 0.2%) and isomenthone (HD = 0.4%; SFME = 0.3%) for HD and SFME oils, respectively (Table 1).

The oils of *Mentha* spp. are known to contain numerous monoterpenoids with limonene, carvone, menthol and menthene as dominating compounds; however, there have been some variations in the constituents from different countries (Hutchings, 1996). The oil of composition of the South African *M. longifolia* has a close resemblance to that of the French chemotype reported by Fraisse et al. (1985).

Analyses of the oil of *M. longifolia* from Italy and Israel revealed piperitenone oxide as the principal component of the oils (Maffe, 1998), while the oil of Sinai variety had 1,8-cineole (28.8%), cis-piperitone oxide (15.4%), and piperitone (13.8%) (Fleisher and Fleisher, 1998).

Hitherto, the main methods used to obtain essential oils from plant materials are hydrodistillation, steam distillation, water distillation, maceration, empyreumatic (or destructive) distillation and expression (Stahl-Biskup and Saez, 2002). Among these methods, hydrodistillation has been the most common approach to extract the essential oils from medicinal plants (Stahl-Biskup and Saez, 2002).

However in order to reduce the extraction time and possibly improve the extraction yield thereby enhancing the quality of the oils and also to reduce the operation costs, new approaches, such as microwave-assisted extraction and pressurized solvent extraction have also been suggested (Kaufmann et al., 2001; Wang and Weller, 2006).

Lucchesi et al. (2004) reported a solvent free microwave method for the extraction of essential oils from three aromatic herbs (basil, garden mint and thyme). According to the authors, the amount of essential oil obtained with this method was more than other extraction methods both from qualitative and quantitative points of view. Our findings in this study has revealed similar trend.

**Toxicity analysis**

The brine shrimp assay of the essential oils of *M. longifolia* showed LC$_{50}$ values of 54.4 and 77.5 µg/ml for SFME and HD, respectively. These values showed that the essential oils of this plant were toxic. However, at 40 µg/ml, which was the least concentration used, the oils were not toxic, as all the brine shrimps survived Figure 1.

The dominance of hydrocarbons in the essential oil probably accounted for the toxicity. The levels of these compounds should therefore be monitored in future assays. This observation is similar to the one made by Fashola et al. (2011). According to some workers, secondary metabolites from plants which are active medicinally are most times toxic to brine shrimp larvae (Aiyeaagbe et al., 2010; Onocha and Ali, 2010).

The lower LC$_{50}$ value of the SFME oil when compared with that of the HD oil in this study proved that the microwave extracted oil was less toxic than the hydrodistilled essential oil; hence, SFME essential oils of *Mentha* spp. may be used for the preservation of processed foods as well as pharmaceutical and natural therapies for the treatment of infectious diseases in humans and animals, while the HD oil due to its high toxicity level should be used less often in folk medicine due to its high antipruritic, carminative, antiseptic and stimulant properties (Al-Rawi and Chakravarty 1988).

**Conclusion**

The presence of compounds, such as α-pinene, 1,8-cineole and piperitone is of importance in pharmaceutical
Table 1. Chemical composition of the oil of *M. longifolia* subsp. *capensis* extracted using hydrodistillation and solvent free microwave extraction methods.

<table>
<thead>
<tr>
<th>S/N</th>
<th>KI</th>
<th>Component</th>
<th>HD (%)</th>
<th>SFME (%)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>940</td>
<td>α-thujene</td>
<td>0.2</td>
<td>0.1</td>
</tr>
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<td>2</td>
<td>945</td>
<td>α-pinene</td>
<td>5.4</td>
<td>3.6</td>
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<td>3</td>
<td>960</td>
<td>Camphene</td>
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<td>4</td>
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<td>Sabinene</td>
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<td>0.1</td>
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<td>5</td>
<td>984</td>
<td>β-pinene</td>
<td>2.4</td>
<td>2.0</td>
</tr>
<tr>
<td>6</td>
<td>992</td>
<td>Myrcene</td>
<td>0.5</td>
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<tr>
<td>7</td>
<td>1003</td>
<td>3-octanol</td>
<td>0.2</td>
<td>t</td>
</tr>
<tr>
<td>8</td>
<td>1018</td>
<td>α-terpenene</td>
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<td>1036</td>
<td>Limonene</td>
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<tr>
<td>10</td>
<td>1047</td>
<td>1,8-cineole</td>
<td>6.5</td>
<td>5.4</td>
</tr>
<tr>
<td>11</td>
<td>1098</td>
<td>Cis-sabinene hydrate</td>
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<td>0.1</td>
</tr>
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<td>12</td>
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<td>15</td>
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<tr>
<td>16</td>
<td>1264</td>
<td>Piperitenone</td>
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<td>17</td>
<td>1346</td>
<td>α-humulene</td>
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<td>18</td>
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<td>β-caryophyllene</td>
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<td>19</td>
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<td>Germacrene D</td>
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<tr>
<td>20</td>
<td>1490</td>
<td>Bicyclogermacrene</td>
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<tr>
<td>21</td>
<td>1615</td>
<td>Caryophyllene oxide</td>
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<td>t</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>96.50</td>
<td>64.60</td>
</tr>
</tbody>
</table>

KI = Kovats index; t = trace. DB - 5MS non-polar column relative to C8 to C24 n-alkanes.

Figure 1. Effects of HD and SFME essential oils on brine shrimps.

industry. The low LC50 values obtained from SFME oil in comparison with HD oil corrobates the safe use of this plant in folk medicine for the relief of minor ailments. The low LC50 value also indicates the possibility of the presence of antitumor and insecticidal compounds (Krishnaraju et al., 2005).
REFERENCES


