ABSTRACT
In this study, the effect of drying procedure on the pigment levels of three traditionally used medicinal plants were determined and the contribution of the pigment compounds to the overall biological potential of these plant species was examined. The contents of total phenols, anthocyanins, carotenoids and chlorophylls a and b were determined spectrophotometrically, in fresh lemon balm (Melissa officinalis L.), marigold (Calendula officinalis L.) and borage (Borago officinalis), as well as in the same microwave dried herbs, freeze-dried herbs and herbs air-dried at room temperature. In order to evaluate the effect of drying conditions on the antioxidant properties of separate pigment extracts, the obtained extracts of carotenoids, chlorophylls, total phenols and anthocyanins were evaluated for their antioxidant capacity using the ABTS radical scavenging assay.

According to the obtained results, different drying techniques affect different bioactive compounds. Fresh herbs were characterized with the highest contents of carotenoids and polyphenols, air-dried herbs exhibited the highest content of anthocyanins, while the highest content of chlorophylls was determined in freeze-dried herbs. Total phenol content of the analysed plants ranged from 10.75 mg GAE/g DM in microwave dried borage to 98.56 mg GAE/g DM in freeze dried lemon balm. The highest content of total carotenoids was determined in fresh marigold (1.41 mg/g DM), freeze dried borage contained the highest content of chlorophylls (7.67 mg/g DM), while air-dried borage contained the highest content of total anthocyanins (17.55 mg/g DM). The results of this study indicated that freeze-drying is the most efficient drying technique in terms of preserving the beneficial bioactive compounds, followed by air-drying at room temperature, which is usually applied in domestic preparation of natural herbal remedies.

Keywords: Antioxidants; drying; medicinal plants; pigments

INTRODUCTION
A great number of studies during the last decade have linked the consumption of medicinal plants with numerous beneficial health properties, leading to increased popularity and renewed interest in exploring the medicinal properties of traditional medicinal plants. The use of traditional medicine has been encouraged by the World Health Organization (WHO) and United Nation Children’s Fund (UNICEF) for its cultural role, greater availability and acceptability than the modern pharmaceutical agents [1]. Herbal supplements are sold in the form of pills, capsules, liquids and creams, but the recent trend has been to take these herbal supplements as „tea“, the common name given to herbal infusions. As more natural remedies are being commercialised, the interest in chemical composition of medicinal herb products is growing because of ongoing developments in nutritional and biochemical surveying.

The therapeutic activity of plants depends on their phytochemical constituents. Plants contain a significant content of tannins, saponins, phytates, fibres, vitamins and minerals. Some phytochemicals such as ascorbic acid and other organic acids have been known to enhance mineral solubility and uptake [2,3]. Naturally derived antioxidants, especially polyphenols, currently present the main focus of scientific community, due to their excellent antioxidant properties, which makes the consumption of foods rich in these compounds highly recommended. Besides the polyphenolic pigments, other biologically active ingredients are present in medicinal plants, especially photosynthetic pigments like chlorophylls and carotenoids. These bioactive compounds have been shown to have possible health benefits with antioxidant, anticarcinogenic, antihypertensive, antimutagenic, and antimicrobial activities [4,5]. Based on the scientific arguments, supplementation of diet with various herbs is recommended among individual consumers, both for its healing properties and nutritive value [6]. Lemon balm (Melissa officinalis L.) and marigold flowers (Calendula officinalis L.) are among the most often used medicinal plants due to their beneficial health effects. The leaves of lemon balm are widely used in Europe as a herbal infusion for their aromatic, digestive and antispasmodic properties in nervous disturbance of
sleep and functional gastrointestinal disorders. Marigold is a herb of ancient medicinal repute. In traditional and homeopathic medicine it has been used for skin complaints, wounds and burns, conjunctivitis and poor eyesight, menstrual irregularities, varicose veins, hemorrhoids, duodenal ulcers, etc. Borage flowers or borage herb are traditionally used as diuretic, diaphoretic, expectorant, anti-inflammatory and as a mild sedative and anti-depressant [7].

Medicinal plants can be used in fresh or dried form. Drying is the most common method for post-harvest preservation of medicinal plants, and must be accomplished as soon as possible after harvesting, to increase the quality of plants and to prevent the expected contamination and losses [8]. Factors such as scale of production, availability of new technologies and pharmaceutical quality standards must be considered for the drying of medicinal plants. Usual domestic drying procedures, i.e. drying without auxiliary energy should only be considered for drying of small quantities. In cases of mass production, the use of technical drying applications is indispensable. The most popular method of drying is convective drying. However, increase in the air temperature usually results in a decrease of the quality of dried herbs [9]. Vacuum microwave drying of food is getting more popular owing to its numerous advantages. The combination of vacuum and microwave technology can overcome many of the shortcomings of the conventional dehydration technologies [10]. Microwaves shorten the drying time compared to conventional methods and leads to a substantial improvement of the final product quality. It has been shown that vacuum microwave dried food can exhibit superior color, rehydration potential, and vitamin content [11,12]. There has been extensive research into microwave drying techniques, examining a broad spectrum of fruits and vegetables including: potato [13], grapes [14], apple [15] and others. However, little data currently exist on microwave drying of medicinal plants.

During drying, enzymatic processes in fresh plant tissues may lead to significant changes in the composition of bioactive constituents of herbs [16]. Especially phenolic compounds, ascorbic acid and pigments responsible for the characteristic green colour of fruits and vegetables, like carotenoids [17,18] and chlorophylls, are highly susceptible to degradation during processing, resulting in colour changes of the material. Therefore, the aim of this study was to determine the effect of three different drying techniques on the content of total phenols, anthocyanins, carotenoids and chlorophylls a and b, in 3 medicinal plants, in order to provide an insight in the levels of polyphenolic antioxidants that are assured by the intake of these plants.

MATERIALS & METHODS

Drying of medicinal plants
Fresh lemon balm leaves (Melissa officinalis L.), marigold flowers (Calendula officinalis L.) and borage plant (Borago officinalis) were collected from the northeastern Croatia in September 2009. The plants were dehydrated by three different methods: freeze-drying (FD), microwave drying (MD), and air-drying at ambient temperature (AD). Freeze drying was conducted in CHRIST freeze dryer (Gamma 2-20) under reduced pressure. A laboratory-scale microwave system (Milestone microwave laboratory systems, 1200 mega microwave processor, Milestone S.R.L., Sorisole, Italy) with a digital balance which recorded the moisture loss was used. During the microwave drying six different microwave output powers were applied (350, 450, 550, 650, 750 and 850 W). Air drying was conducted at ambient temperature of 23-25°C in the dark, in order to avoid the degradation of pigments and polyphenolic compounds. The process of dehydration using all the methods mentioned above was conducted until the moisture content of dried plants was below 12%.

Determination of Total phenol content (TPC) and Total anthocyanins content
Total phenol content of infusions prepared from the analyzed plants (2g/200mL, extracted for 10 min) was determined spectrophotometrically using the Folin-Ciocalteu assay described by Lachman, Hosnedl, Pivec and Orsak [19]. Gallic acid was used as the standard and the results were expressed as mg of gallic acid equivalents (GAE)/g of dry matter (DM) of sample. The total anthocyanin content was determined using the bisulphite bleaching method [20].

Determination of Carotenoid and Chlorophyll content
For the determination of carotenoids, β-carotene and lycopene were determined spectrophotometrically using a method described by Barros et al. [21]. The sum of β-carotene and lycopene represented the content of carotenoids, expressed in mg/g of DM. The content of β-carotene was evaluated using the HPLC analysis, according to the method described by Marković et al. [22]. The chlorophyll content was determined according to a method reported by Huang et al. [23]. The content of chlorophyll was obtained by summarizing chlorophyll a and b contents and the results were expressed in mg/g of DM.
**Determination of antioxidant capacity**

The Trolox equivalent antioxidant capacity (TEAC) of all evaluated extracts (phenols, anthocyanins, carotenoids, chlorophylls) was estimated by the ABTS radical cation decolorization assay [24]. The results, obtained from triplicate analyses, were expressed as Trolox equivalents, and derived from a calibration curve determined for this standard (100-1000 µmol/g of DM).

**RESULTS & DISCUSSION**

According to the results displayed in Table 1, processing of fresh medicinal plants by drying leads to significant fluctuations of bioactive compounds and their antioxidant capacities. No regularity was observed regarding the effect of drying technique and the content of bioactive compounds. Each drying technique affects different bioactive compounds and their antioxidant properties. Generally, the highest content of carotenoids was determined in marigold (1.41 mg/g DM) and borage contained the highest content of chlorophylls (7.67 mg/g DM), as well as the highest content of total anthocyanins (17.55 mg/g DM) due to its purple flowers that are used for its therapeutic activities.

Fresh plants were characterized with the highest contents of carotenoids and polyphenols, with a minor discrepancy in lemon balm. Drying of the plants, by applying the three techniques resulted with a degradation and decrease of both phenolic compounds and carotenoids in all three medicinal plants. Only freeze-dried lemon balm exhibited higher content of polyphenols when compared to fresh plant.

In terms of polyphenols, lemon balm exhibited the highest TPC among the plants (98.56 mg GAE/g DM), followed by borage (28.35 mg GAE/g DM), while marigold exhibited the lowest TPC (24.07 mg GAE/g DM). As can be seen according to the obtained results (Fig. 1), lemon balm is the most abundant source of polyphenolic antioxidants, which was also confirmed with the highest antioxidant capacity of lemon balm extract, when compared to other plants. The highest antioxidant capacity was determined in fresh or freeze-dried plants and then decreased using the different drying techniques.

The content of carotenoids decreased after drying, when compared to fresh plants, which indicates the susceptibility of these beneficial bioactive compounds to degradation at higher temperatures. This was also confirmed by the higher content of carotenoids in freeze-dried plants, where low temperatures are applied during the dehydration of the plant. The biological importance of carotenoids lies in the fact that carotenoids can also serve as antioxidants, and many reports indicated that carotenoids may possess some anticarcinogenic properties, which may be related to their ability to interact with and quench various radical species that can be generated within cells [25]. Therefore the content of ß-carotene was also evaluated using the HPLC analysis, in order to
compare the contents obtained spectrophotometrically and using the HPLC analysis. As can be seen on Fig. 2, considerably higher contents of β-carotene were obtained using the HPLC analysis when compared to the spectrophotometrically determined β-carotene content, which is most likely due to a more efficient extraction technique used for the preparation of HPLC extract of β-carotene. As opposed to the spectrophotometric method, where marigold was evaluated as the richest source of β-carotenoids, the HPLC-obtained results revealed that lemon balm contains significantly higher contents of β-carotene, especially in fresh and freeze-dried plant. Despite the observed discrepancy, correlation analysis confirmed that high correlation exists between the results of both employed methods (r=0.788).

Figure 2. The content of β-carotene in plant extracts determined spectrophotometrically and using the HPLC analysis (μg/g DM)

Although it could be expected that fresh plants contain the highest chlorophylls content, as can be seen in Table 1, the highest content of chlorophylls was determined in freeze-dried plants. This was also observed with carotenoids which were also mostly preserved in the freeze dried plants, when compared to air-dried and microwave dried plants. Interestingly, air-dried plants exhibited the highest content of anthocyanins, a group of polyphenolic pigments which are highly colored substances found in plants and have a great potential of application as both colorants and antioxidants in food, nutraceutical and pharmaceutical preparations for having most of the red, purple and blue colors [26]. According to our results, both freeze-drying and microwave drying lead to a significant deterioration of these compounds, while air-drying at ambient temperature in the dark was the most favorable among the drying techniques. This indicates that anthocyanins may exhibit higher temperature stability during drying and favor moderate drying conditions, in terms of temperature, light and air flow.

Considering the fact that the ABTS radical, used in the ABTS radical scavenging assay reacts with both hydrophilic and lipophilic antioxidants [27], the extracts obtained for the determination of all bioactive compounds was assayed for its antioxidant potential. In general, the extracts obtained for the determination of chlorophylls exhibited the highest antioxidant capacity (0.02-21.01 mmol Trolox/g DM), followed by the extracts of anthocyanins (0.02-1.99 mmol Trolox/g DM) and carotenoids (0.002-0.27 mmol Trolox/g DM) while the aqueous extracts prepared for the evaluation of total phenols provided the poorest antioxidant properties (0.01-0.22 mmol Trolox/g of DM). These results indicate that beside the well known antioxidant capacity of polyphenols, other plant metabolites, especially carotenoids and chlorophylls, widely distributed in medicinal plants, posses good antioxidant properties.

This was also confirmed by the correlation analysis performed for the content of each group of bioactive compounds and the attributing antioxidant capacity. Since the polyphenolic compounds and their antioxidant capacity exhibited the highest linear correlation (r=0.932), followed by carotenoids and their antioxidant
properties \((r=0.751)\), the results confirmed that these groups of bioactive compounds account for the high scavenging ability on ABTS radical.

### Table 1. The content of chlorophylls, carotenoids and anthocyanins and the antioxidant capacity of their extracts determined with the ABTS assay

<table>
<thead>
<tr>
<th>plants</th>
<th>Total chlorophylls</th>
<th>Carotenoids</th>
<th>Total anthocyanins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/g DM</td>
<td>mmol Trolox/g DM</td>
<td>µg/g DM</td>
</tr>
<tr>
<td>Lemon balm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>4.09 ± 0.05</td>
<td>1.32 ± 0.12</td>
<td>972.12 ± 22.31</td>
</tr>
<tr>
<td>AD</td>
<td>3.47 ± 0.61</td>
<td>0.50 ± 0.11</td>
<td>243.09 ± 12.31</td>
</tr>
<tr>
<td>FD</td>
<td>6.12 ± 0.70</td>
<td>0.74 ± 0.06</td>
<td>437.87 ± 10.56</td>
</tr>
<tr>
<td>MD</td>
<td>2.08 ± 0.10</td>
<td>0.08 ± 0.03</td>
<td>338.11 ± 3.19</td>
</tr>
<tr>
<td>Borage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>6.78 ± 0.29</td>
<td>21.01 ± 0.19</td>
<td>1203.54 ± 20.39</td>
</tr>
<tr>
<td>AD</td>
<td>3.59 ± 0.02</td>
<td>0.18 ± 0.01</td>
<td>83.50 ± 9.38</td>
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<tr>
<td>FD</td>
<td>7.67 ± 0.04</td>
<td>0.09 ± 0.00</td>
<td>591.87 ± 17.12</td>
</tr>
<tr>
<td>MD</td>
<td>3.77 ± 0.30</td>
<td>0.04 ± 0.01</td>
<td>151.49 ± 1.19</td>
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<tr>
<td>Marigold</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>4.16 ± 0.27</td>
<td>20.16 ± 0.09</td>
<td>1405.11 ± 44.13</td>
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<td>0.14 ± 0.02</td>
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<tr>
<td>FD</td>
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<td>568.72 ± 10.89</td>
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<tr>
<td>MD</td>
<td>3.21 ± 0.21</td>
<td>0.02 ± 0.03</td>
<td>411.95 ± 4.63</td>
</tr>
</tbody>
</table>

n.d. – not detected

**CONCLUSIONS**

The results of this study provided a better insight in the composition of bioactive compounds of three traditionally used medicinal plants, which beside providing a variety of bioactive compounds, also possess significant antioxidant capacity. The antioxidant capacity of all evaluated bioactive compounds was in agreement with the content of the attributing compounds, thus confirming the high bioactive potential of lemon balm, marigold and borage. Fresh plants were characterized with the highest contents of carotenoids and polyphenols, the highest content of chlorophylls was determined in freeze-dried plants, while air-dried plants exhibited the highest content of anthocyanins. Microwave drying resulted with a significant degradation of all bioactive compounds in the examined plants.

**REFERENCES**


