Total Phenolic Content and Antioxidant Activity of Some Medicinal Aromatic Plants and Their Constituents

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ABSTRACT

The total phenolic content and related radical scavenging activity of essential oils of five medicinal plants Cymbopogon martini, Apium graveolens, Pimpinella anisum, Mentha piperita and Artemisia annua were analyzed. The total phenolics were analyzed by the Folin-Ciocalteau assay while the antioxidant activity of the essential oils was analyzed by the 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH) assay. There was a range of phenolic concentrations in the studied plant essential oils, whose values ranged from 12.69 to 55 μg/g (per 100 g) of gallic acid (GA equivalent) as measured by the Folin-Ciocalteau assay. The essential oils of the seeds of A. graveolens, C. martini, P. anisum, M. piperita and their constituents in the present study.

Keywords: aromatic plant essential oils, pure components, radical scavenging activity, total phenols

INTRODUCTION

Antioxidants are substances which, when added to food products – particularly to lipid containing foods – can increase the shelf life of the food materials. The use of synthetic antioxidants such as Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT) and propyl gallate (PG) have been restricted in foods as these antioxidants are suspected to be carcinogenic (Mahdavi and Salukhe 1995). Therefore, the importance of the search for antioxidants from a natural plant origin has increased in recent years (Singh et al., 2004, 2006). Antioxidants also play an important role in human health. The imbalance between oxidants and antioxidants in our biological system has been suggested to be the cause of ageing and various diseases in human beings. In modern Western medicine, the balance between oxidation and antioxidation is believed to be a critical concept maintaining a healthy biological system (Frankel et al., 1995; Tiwari 2001). A general recommendation to consumers is to increase the intake of fresh food rich in antioxidant compounds (polyphenols, carotenoids) due to their well-known healthy effects. Many plants have been identified as having potential antioxidant activities and their consumption has been recommended (Friedman et al., 1986; Lee and Shibamoto 2000). Bioactive phenols, especially bioflavonoids are very interesting as antioxidants because of their natural origin and ability to act as efficient free radical scavengers (Hetrog et al., 1993, 1995; Langley-Evans et al., 2000). There are reports in the literature where essential oils of the seeds of Mentha piperita and Apium graveolens have shown antioxidant activity (Mimica-Dukic et al., 2003; Pendry et al., 2003). Antimicrobial and insecticidal activities of Cymbopogon martini have also been reported in the literature (Mohan et al., 2003; Prashar et al., 2003). Shakil et al. (2000, 2004) also reported the insect growth regulatory and nematicidal activity of the essential oil from Artemisia annua. Although the hydroxyl radical scavenging activity of Pimpinella anisum has been reported (Murcia et al. 2004) there is no previous report on such activity from the leaves of C. martini and A. annua. The antioxidant activities of water and alcohol extracts of seeds of anise (Pimpinella anisum L) and dill (Anethum graveolens L) were investigated (Mohammad Al-Ismail and Aburjai 2004). In the acidic fractions of Italo-Mitcham black peppermint oil (Mentha × piperita L.), some uncommon phenols, carboxylic acids and lactones have been identified, and reported (Naf and Velluz 1998). In addition selective extraction of oxygenates from savory and peppermint using subcritical water has been reported (Kubátová et al., 2001). Insofar as the antioxidant activity of the leaves of the above plants is concerned, there is no report whatsoever to the best of our knowledge. We thus undertook the measurement of total phenolics and antioxidant activity of plant essential oils of A. annua, C. martini, A. graveolens, P. anisum and M. piperita and their constituents in the present study.

MATERIALS AND METHODS

Chemicals

2,2′-diphenyl-1-picrylhydrazyl radical (DPPH), α-terpineol, limonene, farnesol, p-cymene, linalool and eugenol standards were bought from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Cineole was from Acros (New Jersey, USA) and butylated hydroxyanisole (BHA) was purchased from Thomas Bakers Chemicals, Ltd., Mumbai, India. Folin-Ciocalteau reagent was brought from Sisco Research Laboratories Pvt. Ltd., Mumbai, India and gallic acid (GA) was from Loba Chemie Pvt. Ltd., Mumbai, India. All solvents used were of analytical grade.

Isolation of the oil

The different plant materials were obtained from the National Bureau of Plant Genetic Resources (NBPR), New Delhi. The leaves of A. annua were obtained from the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow. The leaves were dried at room temperature (35°C) till the leaves could be plucked by hand and ground using a domestic model electronic mixer and the powdered plant material were subjected to continuous single hydrodistillation in a Clevenger type apparatus till the oil stopped coming. It took 5 h for completion. The estimated oil yield varied from 0.09 to 0.13%. Essential oils were dried over anhydrous sodium sulphate and the samples were stored at 4°C before use.
Measurement of total phenolics

Total phenolic concentrations were measured using the Folin-Ciocalteau assay (Asami et al. 2003). Briefly, 5 mL of distilled water, 1-5 mL of sample, and 1 mL of Folin-Ciocalteau reagent were mixed and allowed to stand at room temperature. Then, 10 mL of 7% sodium carbonate solution was added, followed by the addition of distilled water filled to volume. Solutions were mixed and allowed to stand at room temperature for 30 min. Total phenolic content was standardized against GA and expressed as μg/100 mg of GA equivalents (GAE). The estimation of phenolic compounds in the essential oils was carried out in triplicate, and the results were averaged.

Antioxidant activity in terms of DPPH assay

Different amounts of various essential oils, pure components (50-250 μL) and BHA (100 ppm, 50-250 μL) were added to different test tubes. Five milliliters of 0.1 mM methanolic solution of DPPH was added to these test tubes and the contents were shaken vigorously. The tubes were allowed to stand at room temperature for 20 min in the dark (Blios 1958). The control was prepared as above without any essential oil, and methanol was used for the baseline correction. The absorbance of the samples was measured at 517 nm by a UV-VIS spectrophotometer Specord 200 Analytik Jena GmbH, Germany. Radical scavenging activity was calculated using the following formula: Percent radical scavenging activity = (Control OD - sample OD)/ Control OD × 100.

Statistical analysis

For the essential oil, three samples were prepared for assays of every antioxidant attribute. The data were presented as the mean ± standard deviation of three determinations (data were not shown). Statistical analyses were performed using a one-way analysis of variance. A probability value of P<0.05 was considered significant.

RESULTS AND DISCUSSION

There was a range of phenol concentrations in the studied plant essential oils analyzed as shown in Table 1. The values varied from 12.69 to 55 μg (per 100 mg of GA equivalent) as measured by the Folin-Ciocalteau assay. It is well known that polyphenols are widely distributed in the plant kingdom and that they are sometimes present in surprisingly high concentrations (Harborne 1993; Catterall et al. 2000; Visioli et al. 2002; Gramaza et al. 2006). Among the medicinal plant essential oils Mentha piperita had the lowest phenolic content (12.69 μg) while Apium graveolens had the highest (55 μg) (Table 1). According to Singleton et al. (1965) various phenolic compounds have different responses in this assay. The structural features of the phenolic compounds (Frankel et al. 1995) may also be responsible for the antioxidant activity of particular essential oils.

Table 1 Phenolic content of different essential oils as per gallic acid standard.

<table>
<thead>
<tr>
<th>Plant material</th>
<th>Total phenolics (μg/100 mg)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artemisia annua</td>
<td>27.33</td>
</tr>
<tr>
<td>Cymbopogon martini</td>
<td>29.32</td>
</tr>
<tr>
<td>Pimpinella anisum</td>
<td>22.00</td>
</tr>
<tr>
<td>Mentha piperita</td>
<td>12.69</td>
</tr>
<tr>
<td>Apium graveolens</td>
<td>55.00</td>
</tr>
</tbody>
</table>

* microgram gallic acid equivalent per 100 g.

Generally, an increase in concentration of essential oil led to the increase of radical scavenging activity for the M. piperita, P. anisum and C. martini essential oils. But this was not true for the other essential oils studied in the present study. For A. graveolens and A. annua essential oils, above a certain concentration (150 μL onwards), radical scavenging activity decreased with an increase in dose concentration. Our report indicates that at a certain concentration (150 μL onwards), the availability of the hydrogen atom to scavenge the DPPH radical might be hindered. It has already been reported that the antioxidant activity of phenolic compounds depends upon their molecular structures, that is, on the availability of phenolic hydrogens and on the possibility of stabilization of the resulting phenoxyl radicals formed by a hydrogen donation (Catherine et al. 1996; Ramathram et al. 1997).

CONCLUSION

The results of the present work indicate that the studied essential oils possess moderate to strong free radical scavenging activity. However, further investigation of individual phenolic and other components, in vivo and antioxidant activity mechanism is warranted. These studies can be useful as a starting point for further applications of these essential oils and their constituents in food and pharmaceutical preparations.
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