HPLC Analysis of Methanolic Extract of Herbs for Quercetin Content

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Herbs are rich source of flavonoids. Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized according to their chemical structure, into flavones, isoflavonoids and flavon-3-ol, anthocyanidins. They have aroused considerable interest because of their potential beneficial effect on human health. They have been reported to have antiviral, anti allergic, anti platelet, anti inflammatory, antitumor, anti carcinogenic and anti ageing properties. HPLC analysis can be used for classification of herbs based upon secondary metabolites. Extract yield at optimum condition was then analyzed by high performance liquid chromatography (HPLC) for quantifying bioactive flavonoid compounds. It was observed that mint contains the highest concentration of quercetin i.e. about 10.8mg/g.

Keyword: Flavonol, HPLC, Quercetin

1. Introduction

Plants occupy a significant place as raw material for important drugs. Plants produce a variety of antioxidants that include carotenoids, flavonoids, cinnammic acid and ascorbic acid, tocopherols to prevent the oxidation of the susceptible substrate (Hollman, 2001). We are mainly concerned with one class of anti oxidants i.e. flavonoids. Over 4000 flavonoids have been identified so far. Flavonoids are essentially ingested through food rather than metabolically synthesized. The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health. They have been reported to have antiviral, anti allergic, anti platelet, anti inflammatory, antitumor, anti carcinogenic and anti ageing properties (Cody et al., 1986; Kuhnau, 1976; Havsteen, 1983; Middleton, 1984).

Flavonoids may help provide protection against these diseases by contributing along with antioxidants vitamins and enzymes. Quercetin is a type of flavonol. The best property of quercetin is that it acts as an antioxidant. (Lakhanpal and Rai, 2007).

1.1 Lemon grass: which belongs to the family Poaceae. The culms (stems) of lemon grass are widely used in teas and other beverages, herbal medicines, and to flavour southeast Asian cuisine, particularly fish stews and sauces. Lemon grass is also grown in pots indoor, to provide rooms with its ‘fresh’ fragrance. Cymbopogon citratus is closely related to C. nardus, the species of grass from which the insect-repellent citronella is derived. The oil extracted from lemon grass has
also been used as an insect-repellent, as well as to perfume beauty products.

1.2 Parsley or garden parsley (Petroselinum crispum): is a species of Petroselinum in the family Apiaceae. It is widely cultivated as an herb, a spice and a vegetable. Parsley is used for urinary tract infections (UTIs), kidney stones (nephrolithiasis), gastrointestinal (GI) disorders, constipation, jaundice, intestinal gas (flatulence), indigestion, colic, diabetes, cough, asthma, fluid retention (edema), osteoarthritis, “tired blood” (anemia), high blood pressure, prostate conditions, and spleen conditions. It is also used to start menstrual flow, to cause an abortion, and as a breath freshener.

1.3 Coriander: an umbelliferous plant indigenous to southern Europe, is found occasionally in Britain in fields and waste places, and by the sides of rivers. It is frequently found in a semi-wild state. Coriander (Coriander Sativum), an umbelliferous plant indigenous to southern Europe, is found occasionally in Britain in fields and waste places, and by the sides of rivers. It is frequently found in a semi-wild state in the east of England, having escaped from cultivation.

1.4 Mint (Mentha canadensis): is a plant that has been used in a variety of cultures, such as India, the Middle East, and Europe. Characteristic of this plant that has a sweet flavor with a cool sensation after eating. Eating mint is good for the body, such as cooling the digestive tract, or if abdominal pain will greatly help relieve the pain. While herbal mint tea drinking may reduce irritable bowel syndrome, stomach cleaning, and also cleans skin disorders such as acne. While cool mint sensation on the skin can help overcome skin irritation. Not only that, mint can also help eliminate toxins in the body and purify the blood by eating them regularly. In fact, crushed mint leaves believed to whiten teeth and eliminate bad breath.

HPLC technique gives a fingerprint analysis of herbs for its components. Various commonly used herbs are a rich source of flavonoids and mainly quercetin which shows good antioxidant properties. In this paper, we have analyzed herbs for their quercetin content.

2. Materials and Methods
Herbs were obtained from Punjab Agriculture University, Ludhiana. Chemicals required: quercetin from Sigma Aldrich, HPLC grade methanol, phosphoric acid, syringe filters(0.45 µm), HPLC grade water.

2.1 Sample Preparation: Herbs were dried and then crushed. Then 1% of extract was prepared in HPLC grade methanol. Then the sample was sonicated using ultrasonicator for 10 min. Then extract was filtered and injected into the HPLC column using mobile phase of 36:64(acetonitrile and 0.1 %phosphoric acid).

2.2 Standard Preparation: Quercetin was used as standard. 100 ppm quercetin standard was prepared run under similar conditions. The wavelength for maximum absorption of quercetin is 365 nm and the flow rate was maintained at 0.5ml / min.

3. Results and Discussion:
It was observed when similar kind of weight of sample was taken the analysed herbs shows the peak area maximum in case of mint. The rest of herbs showed lower content. So mint can be used as a rich source of quercetin from herb.

Table 1.1: shows the peak area and retention time of different herbs and their quercetin content in mg/g:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the herb</th>
<th>Retention time</th>
<th>Peak area</th>
<th>Conc.in mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Lemon grass</td>
<td>15.625</td>
<td>215603</td>
<td>0.12</td>
</tr>
<tr>
<td>2.</td>
<td>Parsley</td>
<td>16.842</td>
<td>7392928</td>
<td>4.20</td>
</tr>
<tr>
<td>3.</td>
<td>Coriander</td>
<td>17.249</td>
<td>8796752</td>
<td>6.10</td>
</tr>
<tr>
<td>4.</td>
<td>Mint</td>
<td>16.913</td>
<td>17700570</td>
<td>10.8</td>
</tr>
<tr>
<td>5.</td>
<td>Quercetin 100ppm</td>
<td>15.967</td>
<td>14541756</td>
<td>100ppm</td>
</tr>
</tbody>
</table>
Fig 3.1: HPLC chromatogram of Methanolic Lemon Grass Extract

Fig 3.2: HPLC chromatogram of Methanolic Parsley Extract

Fig 3.3: HPLC Chromatogram of Methanolic Coriander Extract
4. Conclusion
It was observed that mint showed highest concentration of quercetin. So it can be used as a good source of quercetin. Herbs are enormously important in both traditional and western medicine. Hence it is essential to analyze the phytochemicals present in the plant through a potential technique. Based upon the HPLC fingerprints, it can be concluded that this analytical technique is a convenient method to identify the presence of numerous constituents present in the methanolic extract. The concentration of quercetin in herbs follows the order 10.8 mg/g in mint, 6.15 mg/g in coriander, 4.2 mg/g in parsley and 0.12 mg/g in lemon grass.

5. References