ABSTRACT:

In this study parthenolide content of feverfew product (Tanamigraine capsule) and apigenin content of chamomile products (Chamomill drop, Kamisol solution, Prostatan drop, Prostatan tablet) were determined as effective compound by HPLC. Feverfew product was extracted by methanol/distilled de-ionized water (50:50) and chamomile products by methanol. The HPLC operating conditions were C-18 reversed phase column (VP-ODS 250×4.6 mm, 5 mm), methanol/ distilled de-ionized water (60:40) as the mobile phase at a flow rate of 1 ml/min and UV detection at 214 nm for parthenolide and 2% acetic acid: acetonitril with linear program 80 to 40 % of acetic acid 2% with a flow rate of 1 ml/min and UV detection at 275 nm for apigenin. The amount of parthenolide and apigenin obtained in Tanamigraine capsules and chamomile products is 0.13-0.016% and 0.04-0.35% respectively.

INTRODUCTION

Feverfew (Tanacetum pantheism) is a medicinal herb which includes germacranolides, eg. parthenolide and flavonoids among others. It has traditionally been used in the treatment of migraine, tinnitus, vertigo, arthritis, fever, menstrual disorder, and difficulty during labour, stomachache, toothache and insect bites (1). Sesquiterpene lactones, especially parthenolide are the active component in feverfew (2). Interest in feverfew, however, has dramatically increased since the publication of two double-blind placebo controlled clinical trial in England. These studies clearly established the potential of feverfew in the prophylaxis of migraine (3). The mechanism of migraine prevention by feverfew has not been completely established, many researchers have stated that bioactive component corresponding for the pharmacological functionality is parthenolide (4). Its pharmacological action is similar to that of aspirin. Parthenolide helps to prevent excessive clumping of platelets and inhibits the release of certain chemicals, including cerotoin and some inflammatory mediators (5). The studies also show that parthenolide was effective in inhibition of some tumor cells and pro-inflammatory transcriptional nuclear factors NF-κB (2).

Chamomile (Matricaria recutita) is another herbal medicine and its constituents are coumarins, flavonoids such as apigenin, luteolin and quercetin. Volatile oil, amino acids, and anthemic acid are also among the constituents. Chamomile is stated to possess carminative, antispasmodic and antcatarrhal properties. It has been used for flatulent nervous dyspepsia, travel sickness, nervous diarrhea, and restlessness and especially for gastrointestinal disturbance with associated nervous irritability in children. One of the active compounds identified is apigenin. Apigenin competitively inhibits binding of flunitrazepam to the central benzodiazepine receptor, but lacked activity at other receptors, including muscarinic, α1-adrenoreceptor and GABA1. Greatest antispasmodic activity was exhibited by the flavonoids, especially apigenin which was found to be more than three times as potent as papaverine (1).

In vitro studies have recently determined that at least part of the anti-inflammatory activity of chamomile extracts is due to constituents which inhibit the formation of 5-lipoxygenase and cyclooxygenase and have antioxidant activity. Apigenin and bisabolol appear to be responsible for this activity (6).

Chamomile extract and some constituents demonstrated a dose-dependent antispasmodic effect on isolated guinea-pig ileum. The flavones apigenin, luteolin, patuletin and quercetin demonstrated marked antispasmodic effects. Antispasmodic activity has also been observed with oral administration of apigenin. Apigenin also demonstrated clear anxiomety and slight sedative activity without muscle relaxant in mice. flavonoids are not only adsorbed at the skin surface but penetrate into deeper skin layers (6). Apigenin was also reported to significantly inhibit UV-induced mouse skin tumor genesis (4).

Commercially available herbal preparations of Feverfew (with a claim of “standardized”) were observed to vary widely in their parthenolide content and other percentage of label claim (7). Physiochemical methods were used to measure parthenolide in several commercial feverfew products. The results found a wide variation in parthenolide content and in some products parthenolide was not detected (2). The present study is determination
was aimed at developing a sensitive, precise and accurate HPLC method for the determination of parthenolide and apigenin in some Iranian products and comparing the amount found by the proposed method by the labeled amount.

Materials and Methods

Parthenolide standard (Analytik Co.), apigenin standard (Analytik.Co), HPLC grade methanol (Darmstadt. Merck Co.), distilled de-ionized water, HPLC grade acetonitrile (Darmstadt. Merck Co.), acetic acid 2% (Darmstadt. Merck Co.), Tanamigraine capsule (Zahravi.Co.), Chamomill drop (Amin Co.), Kamsol solution (Darok Co.), Prostatan drop (Isfahan Co.), Prostatan tablet (Isfahan Co.) were purchased from different pharmacies in Tehran.

Chromatographic analysis

The HPLC system consisted of a pump (LC-10AD VP Shimadzu) and C-18 reversed-phase column (VP-ODS 250L 4.6mm). Feverfew was eluted with a mobile phase consisting of methanol and water (60:40) and chamomile with a mobile phase consisting of acetic acid 2% and acetonitrile with linear program 80 to 40 % of acetic acid 2% in 25 minutes with a flow rate of 1 ml/min. The column effluent was monitored with a variable wavelength UV detector at 214 nm for feverfew and at 275 nm for chamomile (SPD-M10VP Shimadzu) which has the ability to scan from 190-800 nm during compound elution. The detector was connected to computer and data were analyzed.

Table 2: Apigenin content of chamomile products

<table>
<thead>
<tr>
<th>Brand name (manufacture)</th>
<th>Label claim apigenin content (%)</th>
<th>Calculated apigenin content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamomill. Drop (Amin Co.)</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Kamsol Solution (Darok Co.)</td>
<td>50-60</td>
<td>35</td>
</tr>
<tr>
<td>Prostatan Drop (Isfahan Co.)</td>
<td>-</td>
<td>9.2</td>
</tr>
<tr>
<td>Prostatan Tablet (Isfahan Co.)</td>
<td>-</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Table1: Parthenolide content of feverfew capsule

<table>
<thead>
<tr>
<th>Brand name (manufacture)</th>
<th>Label claim</th>
<th>Tanamigraine 125 (Zahravi Co.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Label claim</td>
<td>125 mg</td>
<td>125 mg dried of aerial part</td>
</tr>
<tr>
<td>Claimed parthenolide in each capsule (%)</td>
<td>0.2%</td>
<td>0.13-0.16</td>
</tr>
<tr>
<td>Calculated parthenolide in each capsule (%)</td>
<td>0.25-0.5</td>
<td>0.16-0.4</td>
</tr>
<tr>
<td>Direction of the label</td>
<td>1-2 capsule daily</td>
<td></td>
</tr>
<tr>
<td>Expected daily dose parthenolide (mg)</td>
<td>0.25-0.5</td>
<td></td>
</tr>
<tr>
<td>Calculated daily dose parthenolide (mg)</td>
<td>0.16-0.4</td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

Quantitative analysis

In HPLC parthenolide and apigenin peaks in the samples were easily identified by comparing their retention times with the internal standards of parthenolide and apigenin. The samples were injected triplicate. The average of results of each product are given in table 1 and 2.

Sample preparation

The content of 10 Tanamigraine capsules (10 capsules) were weighed and mixed. An accurately weighed portion of this equivalent to 4 capsules was transferred into a volumetric flask containing 10 ml methanol distilled de-ionized water (50/50) and shaken for an hour followed by centrifugation for 15 min at 3000G. The residue was placed in a 10ml volumetric flask to repeat the extraction. The supernatant was filtered through a non-aqueous 0.45 μm filter.

The liquid chamomile products (1 ml Kamsol solution and 5 ml Prostatan drop) were eluted directly by methanol to a final volume of 10ml. 10 Prostatan tablets were grounded with a mortar and pestle and mixed. Equivalent to one tablet was weighed and extracted using 30 ml methanol and shaken four an hour. The mixture was filtered through a non-aqueous 0.45 μm filter. The extraction process was repeated three times.
and the results for apigenin were 0.8-1.6% and 2-8% for intra day and between-day, each respectively.

Determination of method accuracy was performed by injecting triplicate of three different concentrations of standard samples. The recovery value has been 99.5% for parthenolide (concentrations of 0.0625, 0.125, 0.25 mg/ml) and 96-98% for apigenin (concentrations of 0.05, 0.125, 0.25 mg/ml).

DISCUSSION

Tanamigrain capsules claimed 125 mg of dried aerial parts of feverfew with at least 0.2% parthenolide and HPLC analysis revealed 0.13-0.16% parthenolide present, which is not far from labeled amount. The usual standardization level of parthenolide content in feverfew products is 0.2% (2) which is the serve as labeled amount. Table 2 lists label claims for apigenin content and also the amounts determined by HPLC.

Prostatan drop and Prostatan tablet made no claims for apigenin content. There is considerable difference between label claims and calculated amount of apigenin in Chamomile drop and Kamisol solution. Unfortunately, a therapeutic dose for apigenin has not been approved yet. So there is no possibility to compare these two Iranian available chamomile products. Chamomill drop and Kamisol solution, have percentage of apigenin (mg %) given on the label namely 2% and 50-60% respectively.

Our results of apigenin amount in Chamomill drop and Kamisol solution are 12% and 35%. As shown, the calculated content of apigenin in Chamomill drop is six fold higher than its label. But in Kamisol solution the claimed apigenin amount could not be detected. The apigenin amount calculated in Prostatan drop and Prostatan tablet are 9.2% and 0.04% respectively. Unfortunately no measurable data of Apigenin amounts are stated on the label of these two products. There is no possibility to match the obtained results with the label.

REFERENCES:


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