STUDY OF COMPOSITION, ACTIVITY AND PHENOLIC CONTENT OF HERBAL PRODUCTS

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Abstract:
Objective of the study was focused on the formation of novel herbal product from wild herbs and various dry fruits. Herbs are of high medicinal value and dry fruits have antioxidant activities. The ingredients used were Withania somnifera, Piper longum, Asparagus racemosus, Pueraria tuberose, Elettaria cardamomum, Pistacia vera, Citrullus vulgaris, Cucumis melo and Prunus dulcis. These ingredients were tested for proximate analysis for moisture, ash, fat, fiber, proteins and carbohydrates. Apart from these they were also tested individually for their antioxidant activity DPPH method and total phenolic content by Folin-Ciocalteu method. Phenolic content was found to be highest in Piper longum and antioxidant activity in Citrullus vulgaris. These tests were also done on the final product from these compounds. The total fat content and moisture were found to be higher in the product. The final product can be effective as antioxidant, fertility enhancer, anticancer, providing immunity in children and control of cardiovascular problems. The product is of high and effective food value. However, physiochemical studies are required to establish the nature and type of compounds responsible for the bioactivity of the herbal product.

Keywords: Herbs; Herbal Products; Antioxidant Activity and Phenolic Content.

1. Introduction
Man has always been keen to keep himself from all miseries and Mother Nature has provided large number of herbs and spices to fulfill his will. Human history is full of examples of food developed for one or the other health benefit. Greeks used garlic in the first Olympic Games as they considered as a performance enhancing drug. In India, the use of various herbs is as long as the history of mankind. People have used these plants since earliest times. Herbs have charged the course of history and in economic terms they have greater importance as ingredients in food, medicine, perfumery, cosmetics and garden plants (Brown, 1995).

The knowledge of herbs has been handed down from generation to generation from thousands of years. Herbs were woven into the history of nations. They have played a dramatic role in the civilization and history of nations. They are reputed to possess several medicinal and pharmacological properties and hence find position in the preparation of a number of medicines. Even today we continue to depend on herbs for many of our newest medicines, chemicals and flavors and they are used in culinary preparations, perfumery and cosmetics. Many medicinal herbs are also used in food, oil and fiber plants and have always been grown for a range of purposes (Parry, 1969; Rosengarten, 1973; Andi et al., 1997).

Herbs and spices have tremendous importance in the way we live, as ingredients in food, alcoholic beverages, medicine, perfumery, cosmetics and coloring and also as garden plants. Spices and herbs are used in foods to impart flavor, pungency and colour. They also have antioxidant, antimicrobial, pharmaceutical and nutritional properties. In addition to the known direct effects, the use of these plants can also lead to complex secondary effects such as salt and sugar reduction, improvement of texture and prevention of food spoilage. The basic effects of spices, when used in cooking and confectionery can be for flavoring, deodorizing/masking, pungency and coloring. They are also used to make food and confectionery more appetizing and palatable.
Some spices, such as turmeric and paprika, are used more for imparting an attractive colour than for enhancing taste (Ravindran et al., 2002).

Many herbal spices are known as excellent sources of natural antioxidants and consumption of fresh herbs in the diet may therefore contribute to the daily antioxidant intake. Phenolic compounds are the primary antioxidants present in spices and there is a linear relationship between the total phenolic content and the antioxidant properties of spices. Essential oils, oleoresins and even aqueous extracts of spices possess antioxidative properties. Herbs and spices are rich in volatile oils, which give pleasurable aromas. In addition, herbs may contain alkaloids and glycosides, which are of greater interest to pharmacologists. Keeping in view the above mentioned benefits, present study was undertaken with following objectives: a) To prepare a novel product using various herbs and dry fruits. b) To study the chemical and sensory properties of the product to access its quality and acceptability. c) To check the TPC and antioxidant activity of the prepared product.

2. Material and Methods

All herbs and spices (Withania somnifera, Piper longum, Asparagus racemosus, Pueraria tuberosa, Elettaria cardamomum, Pistacia vera, Citrullus vulgaris, Cucumis melo and Prunus dulcis) were purchased from Amritash Herbocare, Ellenabad (India). All herbs and spices were stored in glass jar at room temperature. The ingredients were cleaned to remove foreign materials and the powder was prepared using laboratory grinder (Inalsa Instrument). The powder of each ingredient was screened through 0.150 mesh sieve and was packed in polythene bags until used for further analysis. Other materials like sugar etc. required for herbal product making were purchased from local market. All the chemicals and reagents used were analytical grade.

2.1 Analysis methods for herbs

2.1.1 Chemical analysis

The powder from of herbs and spices were analyzed for their compositions as followed.

a) Crude protein

Crude protein was estimated by Kjeldhal nitrogen estimation method using factor of 6.25 for conversion of nitrogen into protein.

b) Moisture

Moisture content was determined using hot air oven method by taking 5g sample at 100°C temperature for 3 h.

c) Ash

Ash content was determined by taking 5g of sample in a Muffle furnace maintained at 550°C temperature.

d) Crude fat

Crude fat was determined using Soxhlet extraction method using petroleum ether (60-80°C) as organic solvent.

2.2 Procedure

Defatted sample (2g) was weighed in a beaker and added with 200 ml of hot 1.25% H₂SO₄. The mixture was heated to boiling on a crude fiber extraction apparatus for 30 min. The contents were filtered with a Buchner funnel and washed back in beaker with 200ml of 1.25%NaOH. The contents were boiled for 30 min and the material was transferred to a sintered glass crucible. The contents were washed with boiling water and ethanol thrice. The contents were dried in a hot air oven, cooled and weighed. The ashing was done at 550°C for 2h.

a) Carbohydrate

The carbohydrates content was determined by difference method (Kik and Sawyer, 1991).

Chemicals

The organic solvents (methanol) were used for extraction. All other chemicals used like DPPH, Gallic acid, Folin reagent, Ascorbic acid etc. in this study were of Hi Media.

2.3 Determination of total phenolic content (Folin-Ciocalteu method):

Total phenolics content was determined according to the Folin-Ciocalteu method (Folin, 1927) using Gallic acid as standards. Extract powders (1mg) were dissolved in 1ml 50% methanol solution. Extract solution (0.5 ml)
was mixed with 0.5 ml of 50% Folin-Ciocalteu reagent. After 2-5 min, 1.0 ml of 20% Na$_2$CO$_3$ was added to the mixture and incubated for 10 min at room temperature. The mixture was centrifuged at 150 g for 8 min and the absorbance of the supernatant was measured at 730 nm. The total phenolic content was expressed as Gallic acid equivalents (GAE) in milligrams per gram sample.

### 2.3.1 Testing of Antioxidant activity

#### 2.3.2 DPPH radical scavenging assay

5 mg extract was dissolved in 5 ml of methanol solution to obtain 1000 g/ml sample solution. This solution was serially diluted into 10, 20, 30, 40, 50, 70, 80, 90, 100 g/ml with methanol. In each reaction, the solutions were mixed with 1 ml of 0.1 mM 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) and 0.05 ml samples at room temperature for 30 min. Methanol solution was used as negative control. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm. DPPH is a purple-colored stable free radical; when reduced it becomes the yellow-colored diphenylpicrylhydrazine. L-Ascorbic acid was used as positive control. The antioxidant activity of test samples was evaluated by calculating the percent inhibition of superoxide anion radical by applying the following formula

\[
\% \text{ inhibition} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100
\]

Where $A_0$ was the absorbance of the control (blank, without extract) and $A_1$ was the absorbance of the extract. The antioxidant activity of each sample was expressed in terms of IC$_{50}$ (micro molar concentration required to inhibit DPPH radical formation by 50%), calculated from the inhibition curve.

#### Table 1 Ingredients of the herbal product

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount (in grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Withania somnifera</td>
<td>20</td>
</tr>
<tr>
<td>Asparagus racemosus</td>
<td>30</td>
</tr>
<tr>
<td>Pueraria tuberosa</td>
<td>30</td>
</tr>
<tr>
<td>Piper longum</td>
<td>20</td>
</tr>
<tr>
<td>Elettaria cardamomum</td>
<td>20</td>
</tr>
<tr>
<td>Pistacia vera</td>
<td>50</td>
</tr>
<tr>
<td>Citullus vulgaris</td>
<td>40</td>
</tr>
<tr>
<td>Cucumis melo</td>
<td>40</td>
</tr>
<tr>
<td>Prunus dulcis</td>
<td>50</td>
</tr>
<tr>
<td>Sugar</td>
<td>300</td>
</tr>
</tbody>
</table>

### 3 Results and Discussion

The result of the present study have been discussed under following headings:

#### 3.1 Proximate composition of herbs, dry fruits and the herbal product.

#### 3.2 Antioxidant activity of herbs, dry fruits and the herbal product.

#### 3.3 Total phenolic content of herbs, dry fruits and herbal product.

#### 3.4 Sensory evaluation of the herbal product.

#### 3.1 Proximate Composition of Herbs, Dry fruits and the herbal product
The result of proximate analysis shown in Table-2 variant concentration/proportions of biochemical and other content. The moisture content of each species of herbs was different. Looking at overall percentage of moisture content, it was highest in Withania somnifera (15.2%) followed by Asparagus racemosus (13.2%) and Piper longum (11.0%), while Pueraria tuberose (8.8%) has comparatively lesser moisture content. In case of ash content, it was highest in Piper longum (6.4%).

While analyzing the fat contents in the selected herbs, the results showed that Piper longum (17.6%) and Pueraria tuberose have significantly similar and higher fat contents then other herbs. The result of protein analysis showed that there was no much difference in protein content among herbs (ranges between 1.3 to 3.5%). Piper longum has lowest while Pueraria tuberose have highest protein content.

Cude fiber content of these ranges between 3.5 to 5.0%, Pueraria tuberose have highest and Withania somnifera & Asparagus racemosus have similar crude fiber content (3.5%). The carbohydrate content ranges between 59.20 to 64.46%, maximum in Pueraria tuberose and minimum in Piper longum. The proximate analysis of W.domnifera ( moisture 7.30%, ash 3.17%, fat 1.13%, fiber 5.00% protein 0.61% and carbohydrate 24.34%) evaluated by Krishnurthy et al., (2010).

<table>
<thead>
<tr>
<th>In (%)</th>
<th>Withania somnifera</th>
<th>Asparagus racemosus</th>
<th>Pueraria tuberose</th>
<th>Piper longum</th>
<th>Herbal product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>15.2±0.02d</td>
<td>13.2±0.03c</td>
<td>8.8±0.02d</td>
<td>11.0±0.04d</td>
<td>18.0±0.02e</td>
</tr>
<tr>
<td>Ash</td>
<td>3.6±0.02b</td>
<td>2.4±0.02a</td>
<td>1.8±0.02a</td>
<td>6.4±0.03c</td>
<td>1.5±0.03a</td>
</tr>
<tr>
<td>Fat</td>
<td>12.2±0.03c</td>
<td>15.4±0.02d</td>
<td>16.4±0.02e</td>
<td>17.6±0.02e</td>
<td>58.4±0.04g</td>
</tr>
<tr>
<td>Crude Fiber</td>
<td>3.5±0.02b</td>
<td>3.5±0.02b</td>
<td>5.0±0.03c</td>
<td>4.5±0.03b</td>
<td>5.2±0.04b</td>
</tr>
<tr>
<td>Protein</td>
<td>1.60±0.05a</td>
<td>2.30±0.03c</td>
<td>3.54±0.03b</td>
<td>1.30±0.02b</td>
<td>14.2±0.06e</td>
</tr>
<tr>
<td>Carbohydrate*</td>
<td>63.9±0.03f</td>
<td>63.2±0.02e</td>
<td>64.46±0.02f</td>
<td>59.2±0.03b</td>
<td>14.7±0.03c</td>
</tr>
</tbody>
</table>

The values are mean ± S.D. of three independent determinations. The values with different superscripts in a row differ significantly (p<0.05).

*Calculated by difference method.

3.1 b) Proximate analysis of dry fruits

The moisture content of each species of dry fruit was different. Among the overall percentage of moisture content, it was found highest in Cucumis melo (8.6%) followed by Citrullus vulgaris (6.6%) and Prunus dulcis (6.4%) while Pisttscia versa (4.0%) has comparatively lesser moisture content. In case of ash contents, it was highest in Prunus dulcis. While analyzing the fat contents in the dry fruit, the results showed that Pistacia vera (50.4%) and Prunus dulcis (48.2%) have similar and higher fat contents than other dry fruit. The results of protein analysis showed that there was much difference in protein content among dry fruits (ranges 12.4 to 27.8%). Elettaria cardamomum (27.8%) have lowest while Citrullus vulgaris (12.4%) have highest protein content. Crude fiber content of these herbs ranges between 2.0 to 16.0%, Elettaria cardamomum (16.0%) have highest and Cucumis melo (2.0%) have lowest crude fiber content. The carbohydrate content ranges between 16.5 to 42.30%, maximum in Elettaria cardamomum (42.30%) and minimum in Cucumis melo (16.5%). These results were also verified by the study of Akpambang et al., (2008) in C.vulgaris (moisture 4.85%, ash 4.48%, fat 46.24%, fiber 5.00%, protein 25.73% and carbohydrate 13.70%) and in P.ducis (moisture 6.10%, ash 3.34%, fat 42.14%, fiber 5.70%, protein 14.70% and carbohydrate 28.05%) and Hussain et al., (2009) supported the result P.vera (moisture 4.36%, ash 3.26%, fat 51.75%, fiber 3.88%, protein 19.12% and carbohydrate 21.49%)

Table 3 Proximate composition of herbs

<table>
<thead>
<tr>
<th>In (%)</th>
<th>Elettaria</th>
<th>Pistacia vera</th>
<th>Citrullus</th>
<th>Cucumis melo</th>
<th>Prunus dulcis</th>
</tr>
</thead>
</table>
The values are mean ± S.D. of three independent determinations. The values with different superscripts in a row differ significantly (p<0.05).

* Calculated by difference method.

The variations in results may be due to the physiological environment of the sample, human error of handling and variety difference.

3.2 Anti oxidant activity of herbs, dry fruits and the herbal product.

The antioxidant activity of plant extract cannot be evaluated by a single method due to the complex nature of phytochemical present. Therefore, it is important to employ a number of commonly accepted assays to evaluate the antioxidant activities of plant extracts. Numerous antioxidant method have been developed to estimate antioxidant activity but to explain how antioxidants function, DPPH assay is the most commonly accepted ones which also been in the present investigation (Liu et al.,1997; Chen et al.,1999).

Antioxidant activity

Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, inflammation neuro degeneration and ageing process. Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. The result evaluated the free radical scavenger activity of methanolic extract of Watermelon seed (C.vulgaris), Muskmelon seed (C.melo, Pista (P.vera) and Almond (P.dulics). Among the extracts of herbs and standard tested for the in vitro antioxidant activity using the DPPH method, the crude methanolic extracts of Watermelon seed (C.vulgaris), Muskmelon seed (C.melo), Pista (P.vera) and Almond (P.dulics) showed antioxidant activity with different percentage of inhibition as shown in Table-3. The maximum percentage of inhibition was measured in C.vulgaris (97.68%) at 70 g/ml while minimum percentage of inhibition of C.vulgaris was measured (80.20%) at 10 g/ml concentration as compared to positive control i.e. L-ascorbic acid as shown in Table-4. Same as that of Gill et al., (2011) also measured the percentage inchibition of C.vulgaris (79.4%) at 300 g/ml concentration as compared to positive control i.e. L-ascoribe acid. As shown in Fig-2.

In case of C. melo the maximum percentage of inhibition was found (54.67%) at 20 g/ml and minimum was (42.20%) at 40 g/ml concentrations as compared to positive control i.e. L-ascorbic acid. Similar work has been reported by Gill et al., (2011) in C.melo.

In case as shown in Table-4 of herbs the highest percentage of inhibition was determined in P.longum (57.15%) at 70 g/ml while least was in P.tuberosa (27.59%) at 30 g/ml concentration as compared to positive control i.e. L-ascorbic acid as shown in Table1. The maximum antioxidant activity of the herbal product was determined (42.63%) at 70 g/ml and minimum (37.04%) at 20 g/ml concentration Fig -3 as compared to positive control i.e. L-ascorbic acid as shown in Table-3.
Fig-1 Standard L-ascorbic acid

Table-4 Percentage inhabitation of dry fruits and the herbal product

<table>
<thead>
<tr>
<th>Concentration</th>
<th>L-Ascorbic acid</th>
<th>C. vulgaris</th>
<th>C. melo</th>
<th>P. vera</th>
<th>P. ducis</th>
<th>Herbal product</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>29.65</td>
<td>80.206</td>
<td>54.070</td>
<td>38.132</td>
<td>32.734</td>
<td>40.017</td>
</tr>
<tr>
<td>20</td>
<td>36.22</td>
<td>96.658</td>
<td>54.670</td>
<td>34.276</td>
<td>32.734</td>
<td>37.018</td>
</tr>
<tr>
<td>30</td>
<td>44.56</td>
<td>96.230</td>
<td>45.930</td>
<td>36.590</td>
<td>32.991</td>
<td>39.846</td>
</tr>
<tr>
<td>40</td>
<td>55.06</td>
<td>97.087</td>
<td>42.502</td>
<td>36.675</td>
<td>31.277</td>
<td>40.103</td>
</tr>
<tr>
<td>50</td>
<td>62.13</td>
<td>97.515</td>
<td>44.216</td>
<td>40.189</td>
<td>31.448</td>
<td>40.531</td>
</tr>
<tr>
<td>60</td>
<td>70.15</td>
<td>97.001</td>
<td>45.159</td>
<td>36.932</td>
<td>30.934</td>
<td>41.474</td>
</tr>
<tr>
<td>70</td>
<td>77.48</td>
<td>97.686</td>
<td>44.559</td>
<td>35.647</td>
<td>31.277</td>
<td>42.674</td>
</tr>
<tr>
<td>80</td>
<td>83.02</td>
<td>95.887</td>
<td>46.444</td>
<td>36.504</td>
<td>31.620</td>
<td>42.502</td>
</tr>
<tr>
<td>90</td>
<td>89.06</td>
<td>97.344</td>
<td>45.330</td>
<td>38.218</td>
<td>32.476</td>
<td>39.417</td>
</tr>
<tr>
<td>100</td>
<td>94.16</td>
<td>97.087</td>
<td>45.159</td>
<td>37.018</td>
<td>31.362</td>
<td>40.017</td>
</tr>
</tbody>
</table>

Fig-2 Percentage inhabitation of dry fruits and the herbal product

Table-5 Percentage inhabitation of the herbs and the herbal product
3.3 Total phenolic content of herbs and dry fruits

The total phenolic contents of the methanol extract of dry fruits were shown in Table 6. It was found that there was considerable difference in the total phenolic content of selected dry fruits. The maximum total phenolic content was measured in C. vulgaris (18.58 mg/g) as Gallic Acid Equivalent (GAE) and minimum in P. vera (0.84 mg/g) as Gallic Acid Equivalent (GAE). The total phenolic contents of the methanol extract of herbs were presented 4.3.2. It was found that the total phenolic content of selected herbs was also showed heterogeneity respectively. The maximum total phenolic content was found in P. longum (22.8 mg/g) as Gallic Acid Equivalent (GAE). The maximum total phenolic content of the herbal product was measured (16.2 mg/g) as Gallic Acid Equivalent (GAE). The result was verified by the study of Joy et al., (2010) in Piper longum maximum total phenolic compound was measured (7.0 mg/g) as Gallic Acid Equivalent (GAE). It can be revealed from the result that anti oxidant activity is directly related with phenolic content of various extract. Hence higher the phenol contents, higher the antioxidant activity.
3.4 Sensory evaluation of the herbal product

The sensory evaluation of the herbal product was carried out by the sensory panel. Various parameters of sensory evaluation (appearance, colour, taste, texture and overall acceptability) were determined on Hydonic scale. The result of sensory evaluation on Hydonic scale was described in Table 8.

Table-8 Sensory evaluation of the herbal product
References:

[1] Akpambang AOE, Amoo IA and Izuagie AA (2008), Comparative compositional analysis on two varieties of melon (Cucumis melo L) and a variety of almond (Prunus amygdalus) Research, Journal of Agriculture and Biological Sciences: 4(6) 639-642.


