Comparative in vitro antioxidant potential of different spray dried extracts of aerial parts of Sebastiania chamaelea Muell. Arg

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ABSTRACT

Sebastiania chamaelea MUELL. ARG., a medicinal plant from the family of Euphorbiaceae has been selected based on its usage in the traditional, folklore and ethnobotanical importance. In this study, the antioxidant potential of different spray dried extracts of aerial parts of S. chamaelea herb was established. Total phenol content, Total flavonoid content, DPPH radical scavenging activity and reducing power assay of the extracts were determined. IC50 value for free radical scavenging activity of hydro alcohol (Methanol: Water) and alcohol (Methanol) extracts were found to be 0.061 mg/ml and 0.062 mg/ml respectively. The EC50 value for reducing power assay of hydro alcohol and methanol extracts were found to be 0.173 mg/ml and 0.190 mg/ml respectively.

Keywords: Sebastiania chamaelea, Antioxidant activity, Spray drying

INTRODUCTION

Antioxidants have been widely used as food additives to provide protection against oxidative degradation of food [1]. It was evident from the data obtained from various studies that medicinal plants contain a wide variety of natural antioxidants, such as phenolics, flavonoids, and tannins, which possess more potent antioxidant activity than common dietary plants. Compounds responsible for such antioxidant activity can be isolated and used for prevention and treatment of free radical-related disorders [2]. The most commonly used and commercially viable are butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), propylgallate (PG) and butylatedhydroquinone (BHQ). However, there is a rising aversion towards these synthetic molecules due to their potential liver damage and carcinogenesis [3-7].

Sebastiania chamaelea MUELL. ARG., a medicinal plant from the family of Euphorbiaceae has been selected based on its usage in the traditional, folklore and ethnobotanical importance [8-9]. Thorough review of ethnobotanical, ethnopharmacological and modern scientific validation data revealed that not much work has been done on the selected plant in terms of chemical investigation and its bioactivity. However, the family Euphorbiaceae is known for treatment of ailments such as respiratory infections, venereal diseases, toothache, rheumatism, cough, ulcer and wounds [10]. Various classes of chemical constituents have been isolated from different species of Euphorbiaceae, this includes triterpenoids and related compounds (sterols, alcohols and hydrocarbons), phenolic compounds (flavonoids, lignans, coumarins, tannins, phenanthrenes, quinones, phenolic acids, etc.), alkaloids, cyanogenic glucosides and glucosinolates [11].

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The aim of the study is to determine the antioxidant potential of different spray dried extracts of aerial parts of *Sebastiania chamaelea*.

**MATERIALS AND METHODS**

**Collection of plant and authentication**
*Sebastiania chamaelea* MUELL. ARG has been collected from Bannari forest, near Satyamangalam taluk, Erode district in Tamilnadu, India at an altitude of about 450 M in the month of July 2011. The plant material (Accession number- 55151) has been authenticated by bio-repository of medicinal plants, Foundation for Revitalisation of Local Health Traditions, Bangalore, India.

**Equipment**
UV spectrophotometer Model: UV-1800, Make: Shimadzu; Spray dryer, Model: Labultima, LU-222 Advanced equipped with twin cyclones.

**Preparation of extracts**
The dried powder aerial parts of the plant was taken and extracted into respective solvents [methanol and methanol: water (70:30)] by refluxing on an oil bath at 70 °C for 4 hours. The material was filtered and the filtrate was concentrated to 80 % in a rotary evaporator at 45 °C under vacuum. The concentrated extract was spray dried to get dry fine extract in powder form.

**Spray drying**
The concentrated extract was dried using spray drier containing co-current nozzle and twin cyclone.

Spray drying conditions:
- Inlet temperature : 170 °C
- Outlet temperature : 65± 5 °C
- Pump speed : 5
- Atomization pressure : 1.5 bars

**Total Phenols**
The total phenols were determined by Folin – Ciocalteau reagent method described by [12]. The dilute extracts of different concentrations were taken in 10ml glass tubes and total volume made to 3ml with distilled water these are then mixed with 0.5ml. Folin –Ciocalteau reagent (1:1 with water) and 2 ml. Na₂CO₃ (20 %). A blue coloured complex, molybdenum blue developed in each tube, as the phenols undergo a complex redox reaction with phosphomolibdic acid in Folin – Ciocalteau reagent in alkaline medium. The tubes containing the blue solutions were warmed in a water bath for 1 min at 60±5 °C, cooled and absorbance was measured at 650 nm against the reagent blank. The standard curve was prepared using known concentrations of catechol at 650 nm. The total phenol content in the test samples was calculated from the standard curve and expressed as mg. catechol equivalent/ g. sample.

**Total Flavonoids**
Colorimetric aluminum chloride method was used for flavonoid determination [13]. Aliquots of extract were taken and the volume made to 2ml. with methanol and then mixed with 0.1 ml. aluminum chloride (10%), 0.1 ml. Potassium acetate and 2.8 ml. distilled water. The solutions were kept at room temperature for 30 minutes and the absorbance was measured at 415 nm. A standard calibration curve was prepared using known concentrations of quercetin at 415 nm. The total flavonoid in the test samples were calculated from the standard plot and expressed as mg. quercetin equivalent/ g. sample.

**Free radical scavenging activity by DPPH**
The scavenging of DPPH radical was carried out according to the method described by [14]. Various concentrations of the extracts were added to 5 ml of a 0.1mM methanol solution of DPPH. The mixture was shaken and left for 50 min at room temperature in the dark, and the absorbance was then measured with a spectrophotometer at 517 nm. Methanol (1ml) replacing the extract served as control. The percentage of free radical scavenging effect [15] was calculated as follows

\[
\text{Scavenging effect (\%) } = \left[ \frac{A_{517\text{control}} - A_{517\text{test}}}{A_{517\text{control}}} \right] \times 100
\]

Where \(A_{517\text{control}}\) = Absorbance of the control at 517nm
\(A_{517\text{test}}\) = Absorbance of the test at 517nm.
Reducing power assay

The reducing power assay was carried out according to the method described by [16]. Various concentrations of sample in distilled water were mixed with 2.5ml of 0.2M phosphate buffer and 2.5 ml of potassium ferricyanide. The mixture was incubated at 50ºC for 20 minutes. About 2.5 ml of 10% TCA was added and centrifuged for 10 minutes (at 1000g). To 2.5ml of the upper layer, 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride was added and the absorbance was measured in a spectrophotometer (at 700 nm). Higher absorbance of the reaction mixture indicated greater reductive potential.

RESULTS AND DISCUSSION

Total phenol and total flavonoid content was determined along with the in vitro antioxidant activity of different spray dried extracts of aerial parts of S. chamaelea. The results of total phenol and total flavonoid content are given in table I.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Total phenolic content mg. catechol equivalent / g of sample</th>
<th>Total flavonoid content mg. quercetin equivalent/ g of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol extract</td>
<td>154.67 ± 0.51</td>
<td>160.66 ± 1.38</td>
</tr>
<tr>
<td>Hydro alcohol extract</td>
<td>152.66 ± 0.51</td>
<td>98.84 ± 0.52</td>
</tr>
</tbody>
</table>

Each value in the table was obtained by calculating the average of three experiments ± SD

The in vitro antioxidant potential of the herbal extracts using DPPH radical and reducing power was tested with different concentrations of alcohol and hydro alcohol extracts. The percentage scavenging/Inhibitory Concentration (IC₅₀) for DPPH radical scavenging and reducing power absorbance (700nm)/Effective Concentration (EC₅₀) for reducing power activity are given in table II and III respectively.

Table II: Free Radical scavenging activity by DPPH reduction of different spray dried extracts of S. chamaelea.

<table>
<thead>
<tr>
<th>Alcohol extract</th>
<th>IC₅₀ (mg/ml)</th>
<th>Hydro-alcohol extract</th>
<th>IC₅₀ (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (mg/ml)</td>
<td>DPPH reduction inhibition (%) Mean ±SD (n=3)</td>
<td>Concentration (mg/ml)</td>
<td>DPPH reduction inhibition (%) Mean ±SD (n=3)</td>
</tr>
<tr>
<td>Control 0</td>
<td>0</td>
<td>Control 0</td>
<td>0</td>
</tr>
<tr>
<td>0.02</td>
<td>18.03 ± 0.88</td>
<td>0.02</td>
<td>18.68 ± 2.07</td>
</tr>
<tr>
<td>0.04</td>
<td>33.86± 0.93</td>
<td>0.04</td>
<td>32.48 ± 1.65</td>
</tr>
<tr>
<td>0.06</td>
<td>50.73 ± 0.91</td>
<td>0.06</td>
<td>50.77 ± 1.42</td>
</tr>
<tr>
<td>0.08</td>
<td>62.93 ± 0.41</td>
<td>0.08</td>
<td>64.96 ± 1.75</td>
</tr>
<tr>
<td>0.1</td>
<td>76.34 ± 0.93</td>
<td>0.1</td>
<td>78.20 ± 2.01</td>
</tr>
<tr>
<td>BHT #</td>
<td>0.062 ± 0.0007</td>
<td>BHT #</td>
<td>0.094 ± 0.002</td>
</tr>
</tbody>
</table>

#BHT- butylated hydroxy toluene (Reference Standard) IC₅₀ value

Table III: Reducing power activity of different spray dried extracts of S. chamaelea.

<table>
<thead>
<tr>
<th>Alcohol extract</th>
<th>EC₅₀ (mg/ml)</th>
<th>Hydro-alcohol extract</th>
<th>EC₅₀ (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (mg/ml)</td>
<td>Reducing power absorbency (700 nm) Mean ±SD (n=3)</td>
<td>Concentration (mg/ml)</td>
<td>Reducing power absorbency (700nm) Mean ±SD (n=3)</td>
</tr>
<tr>
<td>Control 0</td>
<td>0</td>
<td>Control 0</td>
<td>0</td>
</tr>
<tr>
<td>0.02</td>
<td>0.075 ± 0.002</td>
<td>0.02</td>
<td>0.074 ± 0.019</td>
</tr>
<tr>
<td>0.04</td>
<td>0.118 ± 0.018</td>
<td>0.04</td>
<td>0.139 ± 0.004</td>
</tr>
<tr>
<td>0.06</td>
<td>0.177 ± 0.002</td>
<td>0.06</td>
<td>0.191 ± 0.003</td>
</tr>
<tr>
<td>0.08</td>
<td>0.228 ± 0.006</td>
<td>0.08</td>
<td>0.243 ± 0.011</td>
</tr>
<tr>
<td>0.1</td>
<td>0.280 ± 0.006</td>
<td>0.1</td>
<td>0.298 ± 0.004</td>
</tr>
<tr>
<td>0.2</td>
<td>0.522 ± 0.014</td>
<td>0.2</td>
<td>0.570 ± 0.002</td>
</tr>
<tr>
<td>BHT #</td>
<td>0.094 ± 0.002</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#BHT- butylated hydroxy toluene (Reference Standard) EC₅₀ value

The reducing power activity of different concentrations of alcohol and hydro alcohol extracts and the values are calculated and tabulated as per the method given in [17]. The methanol extract of S. chamaelea showed potent
The antioxidant activity can be attributed to phenol content present in the plant [15,18-21]. Overall there is no significant difference observed in the antioxidant potential of spray dried alcohol and hydro alcohol extracts, however the former would be economically viable. Further evaluation for toxicological parameters is recommended in order to see whether these extracts can replace synthetic antioxidants.

REFERENCE

[1] Nan Wu; Kuang Fu; Yu-Jie Fu; Yuan-Gang Zu; Fang-Rong Chang; Yung-Husan Chen; Xiao Lei Liu; Yu Kong; Wei Liu; Cheng-Bo Gu. *Molecules*, 2009, 14, 1032-1043.