Antioxidant Evaluation of Romasanjanana Lepa- A Compound Formulation for Indralupta

Deodatta Bhadalikar¹, Lavekar G.S.² and Rasika Kolhe³

¹D.Y. Patil University, School of Ayurveda, Nerul, Navi Mumbai, Maharashtra, India
²CCRAS, Department of Shalyatantra, New Delhi, India
³Department of Dravyaguna Vijnana, D.Y. Patil University, School of Ayurveda, Nerul, Navi Mumbai
Maharashtra, India

Publication Date: 24 October 2015


Abstract

Alopecia Areata (AA) is the common form of hair loss affecting the quality of life of many patients. In Ayurved, loss of hair is coined as ‘Indralupta’ described in Kshudra Rogas. Vata Pitta Kapha Dosha along with the vitiation of Rakta is responsible for pathophysiology of Indralupta whereas, according to modern science, Alopecia areata is a tissue-restricted autoimmune disease. Therefore it is important to evaluate the role of antioxidant in the treatment. Ayurveda has advocated Lepa in Indralupta. Therefore a compound formulation consisting of leaf of Karanja, fruit of Kapittha, Kasisa and Hastidant Mashi was formulated following standard guideline. To evaluate the antioxidant activity in the compound formulation, it was subjected to Total phenolic content by Folin’s reagent, Antioxidant by DPPH(1,1-diphenyl-2-picrylhydrazyl), Antioxidant by FRAP (ferric reducing antioxidant power), total flavonoid content, and assessment of phenolic compound by HPLC method on distilled water, methanolic and ethanolic extract of the formulation. Result of the study showed maximum Total phenolic content in distilled water extract, whereas total flavonoid was maximum in methanolic extract, Antioxidant by DPPH was maximum (85.24±0.30) in distilled water extract. Whereas by FRAP method, it was maximum (0.017±0.02) in methanolic extract. Total phenolic content was highest in ethanolic extract.

Keywords Antioxidant; Indralupta; Romasanjanan Lepa

1. Introduction

Alopecia is a chronic dermatological disorder in which people lose some or all of the hair on their head and sometimes on their body as well. It is a chronic inflammatory disease that affects the hair follicles. In Ayurvedic approach, loss of hair is coined out as in term of ‘Indralupta’ under the broad heading of Kshudra Rogas [1]. Overdose of salt also cause Indralupta [2]. Vata Pitta Doshas vitiate the hair follicles and is followed by obstruction of the hair follicles with Sleshma and Shonitha, which restrict their re-growth [1]. Alopecia Areata (AA) is the common form of hair loss affecting the quality of life of many patients. Alopecia has many significant deleterious effects like social anxiety, increased self-consciousness, low – self-esteem embarrassment and depression impairing psychological well-
being thus affecting mental and social status of person [3]. The risk of allopathic treatment outweighs their benefits. The pathophysiology of AA has not been clearly defined; however, it appears as a tissue-restricted autoimmune disease mediated by T lymphocytes [4]. Therefore it is important to evaluate the role of antioxidant in the treatment of AA.

In Ayurveda, ‘lepa’ (local application of medicine) has been advocated in Indralupta’ (Alopecia) [5]. A compound formulation ‘Romasanjana Lepa’ was formulated which consists of leaf of Karanja, fruit of Kapittha, Kasisa and Hastidant Mashi. Phytochemicals such as phenolics, terpenoids, anthocyanins and other flavonoids contributes antioxidant activities in plants. Therefore, Romasanjana Lepa was subjected to evaluate total phenolic content, total flavonoid content, Antioxidant by DPPH (1, 1-diphenyl-2-picrylhydrazyl), Antioxidant by FRAP (ferric reducing antioxidant power) and assessment of phenolic compound by HPLC method.

2. Materials and Methods

Compound formulation ‘Romasanjana Lepa’ consists of leaf of Karanja, fruit of Kapittha, Kasisa, Hastidant Mashi. Tender leaves of Bhringaraja (Eclipta alba Linn. - Compositae) required for Kasisa (Ferrous Sulphate; FeSO₄·7H₂O) Shodhana (purification) and leaves of Karanja (Pongamia glabra Vent; Leguminaceae) were collected from the Dr. D.Y. Patil Ayurvedic Herbal Garden. Kapittha fruit (Feronia elephantum Linn; Rutaceae) and Kasisa (Ferrous Sulphate, FeSO₄·7H₂O) was purchased from the local herbal market. Hastidanta Mashi (Burnt Mashi of Ivory) was purchased from Dindayal Pharmacy. Romasanjanan Lepa was prepared following standard procedure of Lepa described in classical text of Ayurveda. First Kasia was purified with Bhringaraja. Equal quantity of Shuddha Kasisa and Karanja Patra was triturated in till Kalka (homogeneous mixture) formed. Then Swarasa (Fruit pulp juice) of ripen ‘Kapittha’ was added to above Kalka and triturated well till it dried completely. Hastidanta Mashi was added to this dried powder. This powder was then stored in air tight container and named as ‘Romasanjanana Lepa’.

2.1. Extraction of Sample

Ten grams of each sample were suspended in 60 ml of different solvent systems viz; distilled water, methanol, and ethanol and kept overnight [6]. Then the extracts were filtered through muslin cloth and concentrated using rotary evaporator at 56˚C. Total phenolic content, Antioxidant by DPPH, Antioxidant by FRAP, total flavonoid content, and assessment of phenolic compound by HPLC method were carried out on compound formulation Romasanjana Lepa

2.2. Total Phenolic Content by Folin’s Reagent

Folin-Ciocalteu reagent was used for the determination of total phenolic content (TPC) as per. 0.2 ml of the extract was mixed with 0.5 ml of Folin-Ciocalteu reagent and 2ml of 20% aqueous sodium carbonate. The mixtures were incubated for 15 min at room temperature. The absorbance was taken at 650 nm. Total phenolic values were expressed as mg/ml using tannic acid standard which is a common reference compound [7] [8].

2.3. DPPH (1, 1-diphenyl-2-picrylhydrazyl) Antioxidant Assay

The free radical scavenging activity was estimated using DPPH [9]. A solution of 0.3 mM DPPH in methanol was prepared and 2 ml of this solution was mixed with 1.9 ml of distilled water and 100µl of sample dissolved in methanol, ethanol and acetone respectively. The reaction mixture was incubated in dark at room temperature for 30min. The absorbance of the mixture was measured using
spectrophotometrically at 517nm. Readings were repeated in triplicates. The ability to scavenge DPPH radical was calculated using following formulae:

\[
\% \text{ Inhibition} = \frac{\text{Absorbance of the Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100
\]

2.4. FRAP (Ferric Reducing Antioxidant Power) Antioxidant Assay

FRAP assay was carried out according to the method of Benzie and Strain (1996) [10]. FRAP reagent was prepared using acetate buffer (1.6 g sodium acetate and 8 ml acetic acid make up to 100ml) (pH 3.6), 10 mM TPTZ solution in 40 mM HCL and 20 mM iron (III) chloride solution in proportion of 10:1:1 (v/v) respectively. The FRAP reagent was prepared fresh daily and was warmed to 37°C in oven prior to use. A total of 100μl samples extract were added to 3 ml of the FRAP reagent and mixed well. The absorbance was measured at 593 nm using spectrophotometer at 0 mins and after 4 mins. Samples were measured in three replicates. Standard curve of Ascorbic acid was prepared. FRAP reagent was used as a blank both for standard and samples.

FRAP value of sample was obtained using the formula

\[
\% \text{ Inhibition} = \frac{\text{Change in Absorbance of Sample from 0 to 4 mins}}{\text{Change in Absorbance of Standard from 0 to 4 mins}} \times 1000u
\]

2.5. Total Flavonoids Content

Flavonoids were estimated using aluminium chloride solution in methanol. (Zhishen et al., 1999; Zou et al., 2004). The total flavonoids content was determined using a 2% aluminum chloride in methanol solution. The dealcoholized samples were diluted with distilled water in the ratio 1:5. 1.5 ml of diluted samples was taken to which 1.5 ml of AlCl₃ in methanol was added. The samples were then incubated for 10 minutes after which the absorbance was recorded at 368 nm using quercetin as standard. All readings were carried out in triplicates.

2.6. RP-HPLC (Reversed-Phase High Performance Liquid Chromatography) Methodology

Analysis of individual phenolic compound present in the different solvent extracts were performed on a Waters HPLC (Model 2487), using a hypersil C18 reversed phase column 15cm with 5μ particle size. A constant rate of 0.75ml/min was used with two mobile phases: (A) 25% methanol in 1% Acetic acid and solvent (B) 75% methanol in 1% Acetic acid. The elution gradient was linear starting with (A) and ending with (B) over 60 min, using an UV detector set at wavelength 280 nm. Phenolic compound from each sample were identified by comparing their relative retention time with the standards of mixture chromatogram. Standard phenolic compounds were obtained from Sigma (USA). The concentration of an individual compound was calculated on the basis of peak area measurements and then converted to ppm. All the chemicals and solvents used were HPLC spectral grade [11] [7].

3. Observation and Results

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Solvent</th>
<th>Readings (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Distill Water</td>
<td>21.66±0.32</td>
</tr>
<tr>
<td>2.</td>
<td>Ethanol</td>
<td>4.80±1.60</td>
</tr>
<tr>
<td>3.</td>
<td>Methanol</td>
<td>16.05±1.09</td>
</tr>
</tbody>
</table>

Table 1: Estimation of Total Phenolic Content and Antioxidant Activity in Formulated Sample
Total Phenolic Content was maximum in distilled water extract of formulation.

**Table 2:** Estimation of Antioxidant Activity in Formulated Sample by using DPPH Assay (Mean + SD)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Solvent</th>
<th>Readings (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Distill water</td>
<td>85.24±0.30</td>
</tr>
<tr>
<td>2.</td>
<td>Ethanol</td>
<td>70.89±0.05</td>
</tr>
<tr>
<td>3.</td>
<td>Methanol</td>
<td>81.11±0.21</td>
</tr>
</tbody>
</table>

Antioxidant activity in Formulated sample by using DPPH assay was maximum in distilled water extract.

**Table 3:** Estimation of Antioxidant Activity in Formulated Sample by using FRAP Assay (Mean +SD)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Solvent</th>
<th>Readings (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Distill water</td>
<td>0.011±0.01</td>
</tr>
<tr>
<td>2.</td>
<td>Ethanol</td>
<td>0.015±0.013</td>
</tr>
<tr>
<td>3.</td>
<td>Methanol</td>
<td>0.017±0.02</td>
</tr>
</tbody>
</table>

Antioxidant activity in formulated sample by using FRAP assay was maximum in methanolic extract.

**Table 4:** Estimation of Total Flavonoid in the Formulated Sample (Mean+SD)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Solvent</th>
<th>Readings (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Distill water</td>
<td>0.58±0.02</td>
</tr>
<tr>
<td>2.</td>
<td>Ethanol</td>
<td>0.51±0.02</td>
</tr>
<tr>
<td>3.</td>
<td>Methanol</td>
<td>0.72±0.02</td>
</tr>
</tbody>
</table>

Total flavonoid was highest in methanolic extract of formulation.

**Table 5:** Quantification of Various Phenolic Compounds using RP-HPLC

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Gallic Acid (ppm)</th>
<th>Cathechol (ppm)</th>
<th>Caeffic Acid (ppm)</th>
<th>Vanillin (ppm)</th>
<th>p-coumaric acid (ppm)</th>
<th>Ferullic Acid (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distill Water</td>
<td>.........</td>
<td>2.471</td>
<td>0.883</td>
<td>0.337</td>
<td>.........</td>
<td>3.562</td>
</tr>
<tr>
<td>Ethanol</td>
<td>.........</td>
<td>50.701</td>
<td>1.923</td>
<td>0.760</td>
<td>.........</td>
<td>.........</td>
</tr>
<tr>
<td>Methanol</td>
<td>.........</td>
<td>.........</td>
<td>8.066</td>
<td>0.605</td>
<td>.........</td>
<td>.........</td>
</tr>
</tbody>
</table>

4. Discussion

Plants produce various antioxidant compounds to combat reactive oxygen species posing an oxidative stress. Antioxidant activity is strongly dependent on the solvent due to the different antioxidant potentials of phytochemical compounds with distinct polarities and extractability. Antioxidant properties of single compounds within a group can vary remarkably, so that the same levels of phenolics do not necessarily correspond to the same antioxidant responses. Lipid peroxidation is caused due to reactive oxygen species (ROS) which is responsible for the deterioration of food by leading the formation of potential toxic compounds. The concentration of peroxide decreases with the increase in the antioxidant activity, while the absorbance values are much smaller with higher antioxidant activities of the samples. The TBA assay is not specific for malondialdehyde (MDA) which is one of the breakdown products of lipid peroxidation. The non-specificity probably results from the acid eating step of the TBA assay that causes the formation of...
artificial TBA/MDA-like derivatives. The DPPH scavenging activity was found to be in agreement with the % protection activity of the extracts. Correlation analysis clearly determine that assay such as total phenol content, DPPH radical scavenging activity and lipid peroxidation correlates with each other. But total flavonoid content has negative correlation with total phenols and lipid peroxidation [9] [12]. (Table 1)

4.1. Total Phenolic Content

The maximum concentration of the total phenolic was found to be in distilled water 21.66±0.32 mg/ml whereas the minimum concentration was observed in ethanol 4.80±1.60 mg/ml. Distilled water formulation is the better solvent for phenolic content estimation because water molecules can retain the phenolic compounds for a longer period of time [13]. (Table 2)

4.2. DPPH Assay (Antioxidant Activity)

The highest antioxidant capacity of formulation was observed in the distilled water 85.24±0.30 mg/ml and the lowest activity was observed in ethanol 70.89±0.05 mg/ml. Thus the phenolic and polyphenolic compounds are natural antioxidants which enhance the free radical scavenging activity [14]. (Table 3)

4.3. FRAP Assay (Antioxidant Activity)

Ferric reducing antioxidant power was found maximum in methanol 0.017±0.02 mg/ml and minimum in distilled water 0.011±0.01 mg/ml. (Table 3)

4.4. Flavonoids Estimation

Flavonoid content was observed maximum in methanol content 0.72±0.02 mg/ml and minimum in ethanol content 0.51±0.02 mg/ml. (Table 4)

4.5. RP-HPLC

The RP-HPLC results was observed maximum in ethanol formulation were catechol was observed to be 50.701 ppm and caeffic acid 1.923 ppm. Caeffic acid and vanillin in methanol formulation was observed to be 8.066 and 0.605 respectively. (Table 5)

5. Conclusion

Total phenolic content was maximum (21.66±0.32) in distilled water extract, whereas total flavonoid was maximum in (0.72±0.02) methanolic extract, Antioxidant by DPPH was maximum (85.24±0.30) in distilled water extract. Whereas by FRAP method, it was maximum (0.017±0.02) in methanolic extract. In the assessment of Total phenolic content, Catechol (50.701 ppm) and Vanillin (0.760) was maximum in ethanolic extract, whereas caeffic acid was maximum 8.066 ppm in methanolic extract. Result of present study showed that A compound formulation Romasanjanana Lepa consisting leaf of Karanja, fruit of Kapittha, Kasisa and Hastidant Mashi posses antioxidant activity.
References


