Determination of Preliminary Standardization Parameters in Extract of *Hibiscus Rosa Sinensis, Calotropis Gigantea* and Polyherbal Formulation

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Abstract
Hibiscus rosa sinensis, *Calotropis gigantea* and Polyherbal formulation in combination of both the plants extract. the present study is focused on the scientific investigation of the hair growth potential of the herb *Hibiscus rosa sinensis* and its therapeutic efficacy in combination with *C. gigantea*. Formulation has prepared by fusion method, *Hibiscus rosa-sinensis* (HRS) leaves extract (5% w/w), *Calotropis gigantea* (CG) leaf and leaf Polyherbal (HRS+CG, ratio 1:1) total 5% w/w in hydrophilic USP base.

**Key words:** Hibiscus rosa sinensis, *Calotropis gigantean*, Polyherbal formulation

Introduction
The increasing demand for herbal medicines, both in the developing and developed countries has inevitably led to maintaining the quality and purity of the herbal raw materials and finished products. The standardization problem relating to herbal drugs mainly arises from the complex composition of drugs that are used in the form of the whole plant, plant parts or extract obtained there from. To ensure reproducible quality of a herbal remedy, proper control of the starting material is essential. (Mukherjee PK 2002). Some of these preliminary standardization parameters have been attempted on selected plants. *Hibiscus Rosa-Sinensis* a well known member of the family Malvaceae, *Hibiscus rosa-sinensis* grows as an evergreen herbaceous plant. A native to tropical and sub-tropical regions, this plant is extensively cultivated as an ornamental plant (Nadkarni AK et al 1954) *Calotropis gigantea*, commonly known as milkweed or swallow-wort, is a common wasteland weed (Singh et al. 1996). *Calotropis* belongs to Asclepiadaceae or Milkweed or Ak family which includes 280 genera and 2,000 species of worldwide distribution but most abundant in the sub-tropics and tropics, and rare in cold countries. Other familiar plants of *Calotropis* are Milk weed or Silk weed (Asclepias syriaca L.), Butterfly weed (Asclepias tuberosa L.) and Calotropis procera (Ait.).

**Material and Methods:**

**Collection of plant:**
The leaves, of *Calotropis gigantea* and

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Hibiscus rosa sinensis were collected from local regions of Gwalior district. They were further identified for physical characteristics of leaf morphology in Department of Botany, Jiwaji University, Gwalior (India).

**Preparation of extract:**

The powdered leaf material (100 g) was packed into soxhlet apparatus and extracted with organic solvent namely petroleum ether (60-80 °C). The filtrate was evaporated using rotary vacuum evaporator under reduced pressure ≤10 mmHg and extracts were stored in desiccators and used for subsequent experiments.

**Preparation of Test Sample**

The petroleum ether extracts were incorporated into hair cream base prepared by fusion method using o/w base. The cream base consisted of glyceryl monostearate (9%, w/w), light liquid paraffin (20%, w/w), cetyl alcohol (15%, w/w), bees wax (15%), propyl and methyl paraben (0.15%, w/w), glycerol (4.5%, w/w), and water (59%, w/w). A mixture of 5% (w/w) of all the three proportionate extracts HRSF, CGF, and HCF were incorporated in the base to obtain 5% herbal creams (Jain Ritu et al 2011)

**Determination of Physicochemical Constants**

**Ash value**

Ash value helps in determining the quality and purity of a crude drug in powdered form. The total ash usually consists of carbonates, phosphates, silicates and silica. Sulphates present in the drug on long storage get converted in to carbonates and oxide on treatment of drug with conc. H2SO4 the carbonates and oxides get reconverted to sulphate which is stable at high temperature.

**Determination of total ash**

5 gm of the sample was weighed and ignited in silica crucible. The powdered drug is ignited by gradually increasing the heat until carbon free and cool. Thereafter we kept it in desiccators and weighed the total amount of ash. The % of the total ash with reference to the air – dried sample of crude drug was calculated.

**Determination of acid insoluble ash**

Total ash was washed with 25 ml of dilute HCl and transferred in to 100 ml beaker then boiled for 5 min and filtered through an ash less filter paper. The residue was washed with hot water and then the crucible was ignited, cooled and kept in desiccators. The % acid insoluble ash with reference to air – dried sample of crude drug was calculated.

**Determination of water soluble ash**

Total ash was washed with 25 ml of distilled water in to 100 ml beaker. Wire gauze was placed over a bunch burner and was allowed to boil it for 5 min. It was filtered through an ash less filter paper and the residue was washed with hot water. The crucible was ignited on flame, cooled and weighed. The % of water – soluble ash with reference to the dried sample of crude drug was calculated.

**Determination of moisture content**

Moisture is an inevitable component of crude drugs, which must be eliminated as far as it is practicable. The objectives of drying fresh material are to aid in their preservation, protection from enzymatic or hydrolytic reactions, to facilitate subsequent combination and to reduce
their weight and bulk. Since the moisture requirements for enzymatic activity and that which microorganisms demand, vary not only with the species, but also with other environmental factors (e.g., temperature, light etc.), it is difficult to state a precise upper limit of moisture that can be permitted in crude drugs (Mukherjee PK, 2002).

**Determination of Extractive Values**

The amount of extractive a drug yield to a given solvent is often an approximate measure of the amount of a certain constituents which the drug contains. Extractive values indicate the nature of the constituents present in a crude drug.

**Alcohol soluble extractive**

5 gm of air dried coarsely powdered plant material was macerated with 100 ml of 90 % ethanol in a closed flask for 24 hours. It was then shaken frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter filtered rapidly taken precaution against loss of ethanol. 25 ml of filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish. Dried at 105 C and weighed. The % of ethanol soluble extractive was calculated with reference to the air – dried powder.

**Water – soluble extractive**

5 gm of air – dried coarsely powdered plant material was macerated with 100 ml water in a closed flask for 24 hours. It was then shaken frequently during the first 6 hours and allowed to stand for 18 hours. Filtered, evaporate 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish, dried at 105 C and weighed. Calculated the % of water – soluble extractive with reference to the air – dried drug. (Mukherjee, P. K., 2002).

**FORMULATION STUDY**

**Chemical Evaluation**

The prepared formulations were evaluated using standard methods of general characterization, physical and chemical evaluation including Specific gravity, pH, Refractive index, Acid value, Saponification value, Iodine value. (Indian Pharmacopoeia, Vol. II, 1996 & Lehne RK et al 1972)

1. **pH:**

   The determination was carried out at temperature of 25±2⁰. The electrode was immersed in the solution that was examined and measured the pH at the same temperature as for the standard solutions. At the end of a set of measurements, recorded the pH of the solution used to standardize the meter and the electrodes.

2. **Specific gravity:**

   Specific gravity (SG) is a comparison of the density of a substance to the density of water at a specific temperature and air pressure. The standard for the water is 39 degrees F (4 degrees C) at 1 atmosphere of pressure, so water at that temperature and pressure would have a specific gravity of 1. Objects with an SG greater than 1 will usually sink in water, and objects with an SG less than 1 will usually float. The other contributing factor is the surface tension of water, which may prevent objects and substances only a little greater than 1 from sinking.

3. **Refractive index:**

   The refractive index (n) of a substance with reference to air is the ratio of the sine of the angle of incidence
to the sine of the angle of refraction of a beam of light passing from air into the substance. It varies with the wavelength of the light used in its measurement. The temperature should be carefully adjusted and maintained since the refractive index varies significantly with temperature. Commercially available refractometers are constructed for use with white light but are calibrated to give the refractive index in terms of the D line of sodium. To achieve the accuracy, the apparatus was calibrated against distilled water which has a refractive index of 1.3325 at 25°C.

4. **Acid value:** Acid value is the number which expresses in milligrams the amount of potassium hydroxide necessary to neutralize the free acids present in 1g of the substance. Specified quantity of the substance was accurately weighed and dissolved in 50 ml of a mixture of equal volumes of ethanol and ether, previously neutralized with 0.1M potassium hydroxide to phenolphthalein solution. Warmed slowly after connecting reflux the condenser to dissolve the sample. Added 1 ml of phenolphthalein and titrated with 0.1M potassium hydroxide until the solution remains faintly pink after shaking for 30 seconds. Calculated acid value from the expression given below

\[
\text{Acid value} = 5.61n/w
\]

Where \( n \) = the number of ml of 0.1M potassium hydroxide required.
\( w \) = the weight in grams of the substance.

5. **Saponification value:** The saponification value is the number of milligrams of potassium hydroxide necessary to neutralize the free acids and to saponify the esters present in 1g of the substance. The value was calculated as per the method prescribed in Indian pharmacopoeia.

6. **Iodine value:** The iodine value is the number which expresses in grams the quantity of halogen, calculated as iodine, which is absorbed by 100g of the substance under the described conditions. It was determined by the Iodine monochloride method prescribed in Indian pharmacopoeia.

\[
\text{Iodine value} = 1.269 \frac{(a-b)}{w}
\]

\( a \) = number of ml required of an indicator
\( b \) = number of ml required of an indicator repeating
the operation omitting the substance being examined
\( w \) = weight in grams of the substance.

**Results & discussion**

*Calotropis gigantean* is commonly known as Madar and wild growing tropical plant. Human kind first utilized material found in environment on an empirical basis to cure various ailments. Previously it has been reported to contain glycosides, beta-sitosterol, madrine, saponins, alkaloids, tannins, trisacharoides and flavonols. The plant has been used for various disease conditions, including leprosy, ulcers tumors and piles. Various pharmacological activity reported like analgesic activity, anti-infertility, anti-inflammatory activity, hepato protective activity, antimyocardial infarction activity and antidiarrhoeal activity. *Hibiscus rosa sinensis* was selected for the study of prevention of hair fall and hair growth activity of the plant extract.
Hibiscus rosa sinensis is a shrub widely cultivated in the tropics. It is grown as an ornamental plant in gardens throughout India and often planted as a hedge or fence plant. Previous studies have showed that H. rosa sinensis possesses many biological activities, such as antipyretic, analgesic and anti-inflammatory activities. It has also been reported that the plant’s flower possesses antispermatogenic, androgenic, antitumour and anticonvulsant properties. In addition, the leaves and flowers have been found to be aid in the healing of ulcers. The available literature also showed that, the hair growth activity of herbal formulation which includes Eclipta alba Hassk [10 % w/v], Hibiscus rosa sinensis Linn [10 % w/v], Nardostachys jatamansi [5 % w/v] concentration in oil is potential and better results were obtained in animal model study. According to traditional texts (Peter EMJ 2006) it is well accepted that the leaves and flowers of Hibiscus rosa-sinensis have hair growth promoting and anti-greying properties. Moreover, in India the herbal products in the market intended for hair growth include the extract of various parts of Hibiscus rosa-sinensis. Hence, the present study is focused on the scientific investigation of the hair growth potential of the herb Hibiscus rosa sinensis and its therapeutic efficacy in combination with C.gigantea.

The preliminary standardization parameters such as acid insoluble ash, water soluble ash, moisture content, water soluble extractives, Ethyl alcohol soluble extractive and methyl alcohol soluble extractive of the dried plant material is shown in Table1.

Among the formulation study the Evaluation of General Characteristics and Evaluation of physical parameters are discussed in Table 2 & 3 respectively.

**Conclusion:**
All these drugs not only show remarkable activity but are also devoid of potential side effects as compared to synthetic drugs.

**References:**
- Indian Pharmacopoeia 1996, Vol. II
- Mukherjee P. K.(2002): Quality control of herbal drugs, Business Horizons,
- Peter EMJ, Arck PC, Paus R, Hair Growth Inhibition by


Table 1: Determination of preliminary standardization parameters the dried Plant material

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Parameters</th>
<th>(%) <em>H.rosasinensis</em></th>
<th>(%) <em>C.gigantea</em></th>
</tr>
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<tbody>
<tr>
<td>1.</td>
<td>Total ash</td>
<td>10.9</td>
<td>7.2</td>
</tr>
<tr>
<td>2.</td>
<td>Acid insoluble ash</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>3.</td>
<td>Water soluble ash</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>4.</td>
<td>Moisture content</td>
<td>8.4</td>
<td>9.1</td>
</tr>
<tr>
<td>5.</td>
<td>Water soluble extractive</td>
<td>10.6</td>
<td>9.8</td>
</tr>
<tr>
<td>6.</td>
<td>Ethyl alcohol soluble extractive</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>7.</td>
<td>Methyl alcohol soluble extractive</td>
<td>4.6</td>
<td>5.1</td>
</tr>
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</table>
### Table 2: Evaluation of General Characteristics

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Parameters</th>
<th>HRSF</th>
<th>CGF</th>
<th>HCF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colour</td>
<td>Green</td>
<td>Green</td>
<td>Greenish Black</td>
</tr>
<tr>
<td>2</td>
<td>Odour</td>
<td>-</td>
<td>-</td>
<td>Characteristic</td>
</tr>
</tbody>
</table>

### Table 3: Evaluation of Physical Parameters

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Parameters</th>
<th>HRSF</th>
<th>CGF</th>
<th>HCF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Specific gravity</td>
<td>0.9292</td>
<td>0.9383</td>
<td>0.9461</td>
</tr>
<tr>
<td>2</td>
<td>Ph</td>
<td>9</td>
<td>8.3</td>
<td>7.5</td>
</tr>
<tr>
<td>3</td>
<td>Refractive index</td>
<td>1.504</td>
<td>1.472</td>
<td>1.434</td>
</tr>
<tr>
<td>4</td>
<td>Acid value</td>
<td>2.38</td>
<td>2.16</td>
<td>1.9</td>
</tr>
<tr>
<td>5</td>
<td>Saponification</td>
<td>256</td>
<td>257</td>
<td>258</td>
</tr>
<tr>
<td>6</td>
<td>Iodine value</td>
<td>8.73</td>
<td>9.41</td>
<td>10.27</td>
</tr>
</tbody>
</table>