PHYSICO CHEMICAL AND PRELIMINARY PHYTOCHEMICAL ANALYSIS OF UPAKUNCHIKA (Nigella sativa Linn) SEED IN DIFFERENT SOLVENT EXTRACTS

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ABSTRACT

Ayurveda is an ancient system of medicine in India as well as worldwide. Medicinal plants are a source of great economic value all over the world. Nature has given us a very rich botanical wealth and large number of diverse types of plants in the world. Among various medicinal plants, Upakunchika i.e., Nigella sativa is a miracle herb with a rich historical and religious background. Present study was designed to perform the Physicochemical and Preliminary Phytochemical analysis of Upakunchika i.e. Nigella sativa seeds. Phytochemical analysis helps in knowing pharmacological actions. Different parameters like Total Ash value, Acid insoluble Ash value, Moisture content, extracts in different solvents and Preliminary Phytochemicals of Nigella sativa seeds were evaluated.

Keywords: Nigella sativa, Physicochemical, Preliminary Phytochemical analysis, Upakunchika

INTRODUCTION

Nigella sativa is the most popular medicine throughout the world. It belongs to the family Ranunculaceae, commonly known as Black seed. It grows in Mediterranean countries and Asian countries including India, Pakistan, Indonesia, Italy and Afghanistan. In India it is called as Kalonji. The properties attributed are Katu, Tikta Rasa, Katu Vipaka, Ushna veerya, Ruksha, Teekshna, Dipana, durgandha nashana, pittakara,. Medhya, Garbhasaya visuddhakrit, Jwaragha, Pachana, Vrishya, Balya, Ruchyam, KaphaVataapaham, Chakshushya. The thera-
are trigonous, angular, rugulose tubercular, 2-3.5×1-2 mm, black externally and white inside, aromatic. Useful part is seed. It has different pharmacological activities like Anti bacterial, Anti fungal, Estrogenic, Anti cancer, Galactogogue, Anti fertility, Anti inflammatory and Analgesic. As Nigella sativa Linn. is a miracle herb, there is a need to analyze its physico chemical and phytochemicals. Upakunchika is one of the easily available, cost effective drug and having many actions against different diseases. So, the drug was selected for the study. The Physico chemical analysis gives us knowledge and proof regarding quality. Preliminary Phytochemical screening will help in knowing various phytochemicals present in nigella seed which are responsible for different pharmacological activities. Different solvents are used to know in which solvent phytochemicals are more available thus it gives further scope in research side. Here an attempt is made to evaluate the Physico chemical and Preliminary Phytochemicals of Upakunchika i.e., Nigella sativa seeds.

AIMS AND OBJECTIVES
1. To evaluate the Physico chemical analysis of Upakunchika.
2. To analyze the Preliminary Phytochemical screening of Nigella sativa Linn. i.e., Upakunchika in different solvents.

MATERIALS AND METHODS

Collection of Plant Material:
Seeds of Upakunchika i.e. Nigella sativa Linn. were collected from Dr. B.R.K.R Govt. Ayurvedic College Garden, Erragadda, Hyderabad and authenticated by Professor & HOD, Department of Dravyaguna and Telangana state level Drug Testing Laboratory, Hyderabad.

Preparation of powder:
Collected seeds were washed under running tap water to eradicate dust and microbes. The seeds were then dried under shade at room temperature. The seeds were crushed well into fine powder in an electronic grinder and kept into air tight polythene bags for further use and stored at room temperature.

PHYSICO CHEMICAL ANALYSIS

1) Foreign matter Drugs should be free from moulds, insects, animal faecal matter and other contaminations such as earth, stones and extraneous material. Foreign matter is material consisting of any organ or part of organ, other than

Fig- 1 Upakunchika plant

Fig- 2 Upakunchika seeds
those named in the definition and description. The amount of foreign matter shall not be more than the percentage prescribed in the monograph of API.

**Determination of Foreign Matter**
Weigh 100 –500 g of the drug sample to be examined or the minimum quantity prescribed in the monograph, and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use of a lens (6x). Separate and weigh it and calculate the percentage present.

2) **Determination of Total Ash:**
Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450° until free from carbon, cool and weigh.

3) **Determination of Acid Insoluble Ash:**
Boil the Ash obtained in (2) for 5 minutes with 25 ml of Dilute Hydrochloric acid, collect the insoluble matter in a Gooch crucible, or on an ashless filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble Ash with reference to the air dried drug.

4) **Determination of Alcohol Soluble Extract:**
Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of Alcohol of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

5) **Determination of Water Soluble Extract:**
Proceed as directed for the determination of Alcohol-soluble extractive, using chloroform water instead of ethanol.

6) **Determination of Moisture Content (Loss on Drying):**
Place about 10 g of drug after accurately weighing it in a tared evaporating dish. After placing the above said amount of the drug in the tared evaporating dish dry at 105° for 5 hours, and weigh. Continue the drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccators, show not more than 0.01 g difference.

**PHYTO CHEMICAL ANALYSIS**

**Chemicals used:**
Methanol, Ethanol, Petroleumether, Chloroform, HCL, Mayer’s reagent(Potassium Mercuric Iodide), Benedicts’ reagent, FeCl3, Benzene, Ammonia, H2SO4, FeCl3, Leadacetate, Ninhydrin reagent, Copperacetatesolution.

Extractive values of *Nigella sativa Linn.* with different solvents is determined with the specific standard methods explained Ayurvedic Pharmacopoeia of India.5

**Solvent extract of sample:**
The extracts of sample powder were prepared by soaking 5 gm of dried powder in 100 ml of each methanol, ethanol, petroleum ether, chloroform and water and shaken well. The solution left at room temperature for 72 hours and then filtered with the help of filter paper. The filtrate was taken and used for further phytochemical screening6.
Physicochemical screening:

1. **Detection of Alkaloids:**
   Extracts were dissolved individually in dilute Hydrochloric acid and filtered.
   **Mayer’ Test:** Filtrates were treated with Mayer’s reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

2. **Detection of Carbohydrates:**
   Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.
   **Benedict’ Test:** Filtrates were treated with Benedict’s reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

3. **Detection of Glycosides:**
   Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.
   **Modified Borntrager’s Test:** Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of Benzene. The Benzene layer was separated and treated with Ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

4. **Detection of Saponins:**
   **Foam Test:** 0.5 gms of extract was shaken with 2ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. **Detection of Phytosterols:**
   **Salkowski’s Test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of con. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

6. **Detection of Phenols:**
   **Ferric Chloride Test:** Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

7. **Detection of Tannins:**
   **Ferric Chloride Test**
   Extracts were treated with 3-4 drops of ferric chloride solution. Formation of green colour indicates the presence of tannins.

8. **Detection of Flavonoids:**
   **Lead acetate Test:** Extracts were treated with few drops of lead acetate solution. Formation of yellow coloured precipitate indicates the presence of flavonoid.

9. **Detection of Aminoacids:**
   **Ninhydrin Test:** To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acids.

10. **Detection of Diterpenes:**
    **Copper acetate Test:** Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.
RESULTS

Table 1: Physico Chemical analysis of Upakunchika

<table>
<thead>
<tr>
<th>S.No</th>
<th>Characteristics</th>
<th>Values</th>
<th>API Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Foreign matter</td>
<td>2% w/w</td>
<td>Not more than 2%</td>
</tr>
<tr>
<td>2.</td>
<td>Total Ash</td>
<td>4.06%</td>
<td>Not more than 6%</td>
</tr>
<tr>
<td>3.</td>
<td>Moisture content</td>
<td>6.1%</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Acid insoluble Ash</td>
<td>0.059%</td>
<td>Not more than 0.2%</td>
</tr>
<tr>
<td>5.</td>
<td>Water soluble extract</td>
<td>10.33%</td>
<td>Not less than 15%</td>
</tr>
<tr>
<td>6.</td>
<td>Alcohol soluble extract</td>
<td>26.54%</td>
<td>Not less than 20%</td>
</tr>
</tbody>
</table>

TABLE 2: Preliminary Phytochemical Analysis of Upakunchika

<table>
<thead>
<tr>
<th>Chemical constituents</th>
<th>Upakunchika</th>
<th>Aqueous extract</th>
<th>Ethanol extract</th>
<th>Methanol extract</th>
<th>Ether extract</th>
<th>Chloroform extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tri terpenoids</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Diterpenes</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Gallo Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Amino acids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

+ sign indicates presence, - sign indicates absence

DISCUSSION

Nigella sativa is a well-known medicinal plant known to have health benefits against many diseases. These health benefits are mainly accounted to the presence of many active phytochemicals in its seed.

PHYSICOCHEMICAL ANALYSIS:

The physicochemical analysis mainly evaluates the quality of the drug. More the quality more will be the therapeutic effect. Here, the analytical results of Upakunchika seeds showed that the foreign matter 2% w/w, Moisture content is 6.1%, Total Ash is 4.06%, Acid insoluble Ash is 0.059%, Water soluble extractive is 10.33% and Alcohol soluble extractive is 26.54%. By observing the above values it shows that in Upakunchika, the foreign matter, Total Ash & Alcohol soluble extractive values and acid Insoluble Ash are within the values mentioned in API. But the value of water soluble extractive value is less than API standards it may be due to potency of the drug depends on the season, time of collection, place of collection. It also showed that Alcoholic extractive value is more than Water extractive value which suggest that it is more soluble in alcohol than in water.

PRELIMINARY PHYTOCHEMICAL ANALYSIS:

Phyto-chemicals present in Aqueous extract of Upakunchika seed powder are Saponins, Glycosides, Phenols, whereas Glycosides,
Phytosterols, Tannins are present in Ethanolic extract. In Methanolic extract, Diterpines, Saponins, Glycosides are present. In Ether extract, Glycosides, Gallo Tannins are present. In Chloroform extract, Glycosides, Saponins, Gallo Tannins are present. Glycosides are present in all four extracts.

Phyto pharmacological studies have shown that, all these phyto chemicals have been found exhibiting Analgesic and Anti-inflammatory, anti oxidants etc properties hence, it can be used in pain management, general debility etc.

CONCLUSION

Upakunchika i.e., Nigella sativa Linn. is very popular drug. The physico chemical analysis helps in assessing the quality of the drug where as the phytochemical analysis helps to evaluate pharmaco therapeutics of the drug. The Physicochemical values of Upakunchika were nearer to API values except Water soluble extract. The Preliminary Phytochemicals like Saponins, Glycosides, Phenols, Phytosterols, Tannins, Diterpines and Gallo Tannins are present in different extracts of Upakunchika.

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