Pharmaceutical Standardization
Pharmacognostical and analytical study of Tulsi-Amla-Yasti Ghrita

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Abstract

_Tulasi Amla Yashti Ghrita_ is an Ayurvedic formulation, which is beneficial in the management of the side effects of Head and Neck Malignancies induced by Radiotherapy and Chemotherapy. A pharmacognostical study involving both the macroscopic and powder microscopy of raw drugs of _Tulasi Amla Yashti Ghrita_ and a physicochemical analysis of the finished product were carried out, to evaluate the quality of the formulation. The specific gravity of the formulation was 0.9130 and pH was 3.5. Thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC) were carried out after organizing the appropriate solvent system, in which five spots were distinguished in TLC and nine spots in HPTLC. Most of the Rf values for the spots observed were identical. The observations could be considered to be the reference standards in future studies.

Key words: Chemotherapy, high performance thin layer chromatography, radiotherapy, _Tulasi Amla Yashti Ghrita_

Introduction

The vital responsibility of herbal medicine in serving the therapeutic requirements of the human populace worldwide has been identified from ancient times to the modern era. However, a key obstacle that has hindered the acceptance of alternative medicines in developed countries is the lack of documentation and rigorous quality control. A need is always felt for the proper documentation of research studies carried out on traditional systems of medicine. Keeping this in mind, it becomes extremely important to develop the standards of plant-based medicines.

For accurate identification, authentication, and standardization of herbal medicines, pharmacognosy is important and necessary. _Tulasi Amla Yashti Ghrita_ (TAYG) is one of the most potential formulations, with some modification in the previous _Anubhuta Yoga_, which is claimed to be effective in the management of complications induced by Radiotherapy and Chemotherapy.[1] Cancer is the most dreaded disease spreading with continuance and increasing in the twenty-first century. It is becoming the most common cause of death in the world.[2] In India, Head and Neck Cancers (HNCA) account for 30-40% of the cancers of all sites.[3] Tobacco- and smoking-related oral cancer accounts for about 33%. Ayurveda, the Indian traditional system of medicine, has many options in this regard, and thousands of combinations remain unexplored. TAYG is one among them. The formulation taken is an _Anubhuta Yoga_. The objective of the study is to develop possible quality control parameters of the drug.

Materials and Methods

The dried fruits of _Amalaki_ ( _Emblica officinalis_ Gaertn.) were collected from the local market of Ahmedabad, Gujarat, and _Tulasi_ ( _Ocimum sanctum_ Linn.), _Yastimadhu_ ( _Glycyrrhiza glabra_ Linn.), and _Goghrita_ (Cow ghee) were procured from the local market of Jamnagar, Gujarat. The herbal material was identified for its genuinity in the Pharmacognosy Laboratory of the Institute. Physical impurities were removed from the herbal drugs, and they were dried below 45°C, and made into a coarse powder to use for the pharmacognostical study. _Tulasi Amla Yashti Ghrita_ [Table 1] was prepared as per the classical reference. A physicochemical analysis of the final product was carried out in the Pharmaceutical Chemistry Laboratory of the institute.

**Macrosopy**

The raw drugs and powders were separately studied by

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organoleptic and morphological characters like Rupa (Color), Rasa (Taste), Gandha (Odor), Sparsha (Touch), and so on.

**Microscopy**

The powders of the drugs were studied microscopically and the characters were observed after proper mounting and staining with Phloroglucinol and HCl (used for identification of Lignified elements). Photographs of the microscopical powder characters were taken using a Canon digital camera attached to a Zeiss microscope, in the Pharmacognosy Laboratory.

**Physicochemical parameters and phytochemical analysis**

Tulasi Amla Yashti Ghrita was subjected to an examination of the physicochemical parameters, to evaluate the possible analytical profile.

**Oraganoleptic characters**

Oraganoleptic characters like Rupa (Color), Rasa (Taste), Gandha (Odor), and Sparsha (Touch) were observed.

**Physicochemical parameters**

Tulasi Amla Yashti Ghrita was subjected to an examination of the physicochemical parameters like refractive index, specific gravity, pH value, acid value, saponification value, and iodine value.

**Thin layer Chromatography**

Thin Layer Chromatography and High Performance Thin Layer Chromatography were performed for the phase separation of the components of unsaponifiable fraction of TAYG. The spots obtained from both the extracts were examined under ultraviolet light, of wavelengths 254 nm and 366 nm.

- Sample preparation:
  - Track 1: Methanolic extract of TAYG
  - Track 2: Unsaponifiable matter of TAYG
- Solvent system:
  - Toluene: Ethyl acetate: Formic acid: Glacial acetic acid (5:5:1:1)
- Stationary phase:
  - Silica gel G
- Visualization:
  - Under long UV (366 nm) and short UV (254 nm)

**High performance thin layer Chromatography**

The HPTLC study of the unsaponifiable fraction of TAYG was carried out by using the same solvent system of Toluene: Ethyl acetate: Formic acid: Glacial acetic acid (5:5:1:1). After completion of HPTLC; post chromatographic derivation was done with methanolic sulfuric acid.

**Results**

Rupa (color), Gandha (odor), Rasa (taste), and Sparsha (texture) of the composition of the formulation can be seen in Table 2.

All the ingredients of TAYG can be seen in Figures 1-3. Powder microscopy of Ocimum sanctum (Sweta Tulasi) revealed the presence of prismatic crystals of calcium oxalate [Figure 4a], oil globules [Figure 4b], annular vessels [Figure 4c], stomata [Figure 4d], spiral vessels [Figure 4e], group of lignified fibers [Figure 4f], a multicellular simple trichome [Figure 4g], and a unicellular simple trichome [Figure 4h]; whereas, microscopy of the coarse powder of the pericarp of Amalaki (Emblema officinalis Gaertn.) showed a prismatic crystal with parenchyma cells [Figure 5a], starch grains [Figure 5b], scleroid [Figure 5c], and a group of lignified fibers [Figure 5d]. The microscopical features seen in the powder of the rhizomes of Glycyrrhiza glabra Linn. (Yastimadhu) were cork cells [Figure 6a], simple and compound starch grains and prismatic crystals of calcium oxalate [Figure 6b], crystal fibres [Figure 6c] and bordered pitted vessels [Figure 6d]. The diagnostic features observed by powder microscopy were authenticated by comparing the features with the standard references.

The drug TAGY is semi-solid in form, brownish chocolate in color, nonspecific in taste, with an aromatic odor. The results observed through the physicochemical parameters can be found in Table 3. The results of TLC can be found in Table 4 and Figure 7; while the results of HPTLC can be found in Table 5 and Figure 8.

**Discussion**

The present formulation consisted of three plant ingredients, which were proved to be genuine, by assessing the organoleptic

| Drug     | Botanical name          | Part used   |
|----------|-------------------------|-------------|
| Amalaki  | Emblica officinalis Gaertn. | Pericarp    |
| Yastimadhu | Glycyrrhiza glabra Linn.   | Rhizome     |
| Sweta Tulsi | Ocimum sanctum Linn.     | Leaves      |
| Go Ghrita – Cow milk ghee | -              | -           |

**Table 2: Macroscopic characters**

| Drug     | Part used | Nature of powder | Color          | Taste              | Odor       |
|----------|-----------|------------------|----------------|--------------------|------------|
| Yastimadhu | Rhizomes  | Coarse           | Brownish-yellow | Sweet, bitter      | Sweetish   |
| Amalaki  | Pericarp  | Coarse           | Grayish-green  | Astringent, sour   | Characteristic |
| Sweta Tulsi | Leaf     | Coarse           | Green          | Pungent, slightly bitter, astringent | Characteristic aromatic |

**Table 3: Physicochemical parameters**

| Test                | Result                      |
|---------------------|-----------------------------|
| Refractive index    | 1.4710                      |
| Specific gravity    | 0.9130                      |
| pH value            | 3.5 (by pH indicator paper) |
| Acid value          | 4.3968 w/v                  |
| Saponification value| 209.82 w/v                  |
| Iodine value        | 23.04 w/v                   |

| Table 1: Formulation composition of Tulasi Amla Yashti Ghrita |

| Drug     | Botanical name          | Part used   |
|----------|-------------------------|-------------|
| Amalaki  | Emblica officinalis Gaertn. | Pericarp    |
| Yastimadhu | Glycyrrhiza glabra Linn.   | Rhizome     |
| Sweta Tulsi | Ocimum sanctum Linn.     | Leaves      |
| Go Ghrita – Cow milk ghee | -              | -           |
characters and powder microscopy features. Physicochemical parameters were applied for assessing the prepared formulation. The refractive index was 1.4710. The specific gravity of the sample was 0.9130, which was closer to plain Ghrita, for which it was 0.9, showing that the sample was not too dense. The pH of TAGY was found to be 3.5, showing the acidic nature of the drug. The acid value was 4.3968 w/v, indicating the amount of free fatty acid present in the Ghrita. The saponification value was found to be 209.82 w/v. It gave an idea of the molecular weight of an oil/fat, and the oil contained a long chain of fatty acids.
acids. The observed iodine value for the sample was 23.04 w/v, which indicated the consumption of the iodine molecules by free fatty acids. TLC showed five spots, when the plate was scanned at 254 nm and 366 nm. The results were the same for both the detection wavelengths, which showed that the components were sensitive to both wavelengths.

**Conclusion**

The preliminary organoleptic features and the results of powder microscopy revealed the presence of important characters like oil globules, a multicellular glandular trichome, multicellular and unicellular simple trichomes in *Tulasi*, prismatic crystal with parenchyma cells, and tannin content in *Amalaki*, and simple and compound starch grains, crystal fibers, and bordered pitted vessels in *Yastimadhu*. Thin layer chromatography results showed five spots when the plate was scanned at 254 nm and 366 nm. The physicochemical analysis showed specific gravity (0.913), pH (3.5), acid value (4.3968), iodine value (23.04 w/v), and saponification value (209.82 w/v). All the parameters
discussed here could be used as identifying tools for the quality assessment of Tulsi Amla Yashti Ghrita.

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