



Pharmaceutical Standardization

Pharmacognostical and analytical study of *Tulasi-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 R_f 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* (*Embllica officinalis* Gaertn.) were collected from the local market of Ahmedabad, Gujarat, and *Tulasi* (*Occimum 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. The 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.

Macroscopy

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).^[4] 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.

Organoleptic characters

Organoleptic 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.^[5]

Thin layer Chromatography

Thin Layer Chromatography and High Performance Thin Layer Chromatography^[6,7] 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^[6,7] 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 TAGY can be seen in Figures 1-3. Powder microscopy of *Occimum 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* (*Embllica officinalis* Gaertn.) showed a prismatic crystal with parenchyma cells [Figure 5a], starch grains [Figure 5b], sclereid [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.^[8-10]

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

Table 1: Formulation composition of Tulasi Amla Yashti Ghrita

Drug	Botanical name	Part used
<i>Amalaki</i>	<i>Embllica officinalis</i> Gaertn.	Pericarp
<i>Yastimadhu</i>	<i>Glycyrrhiza glabra</i> Linn.	Rhizome
<i>Sweta Tulsi</i>	<i>Ocimum sanctum</i> Linn.	Leaves
<i>Go Ghrita</i> –Cow milk ghee	-	-

Table 2: Macroscopic characters

	<i>Yastimadhu</i>	<i>Amalaki</i>	<i>Sweta Tulasi</i>
Part used	Rhizomes	Pericarp	Leaf
Nature of powder	Coarse	Coarse	Coarse
Color	Brownish-yellow	Grayish-green	Green
Taste	Sweet, bitter	Astringent, sour	Pungent, slightly bitter, astringent
Odor	Sweetish	Characteristic	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



Figure 1a: *Tulsi* leaves



Figure 1b: *Tulsi* leaf powder



Figure 2a: *Amalaki* fruit



Figure 2b: *Amalaki* fruit powder



Figure 3a: *Yashtimadhu* root



Figure 3b: *Yashtimadhu* powder

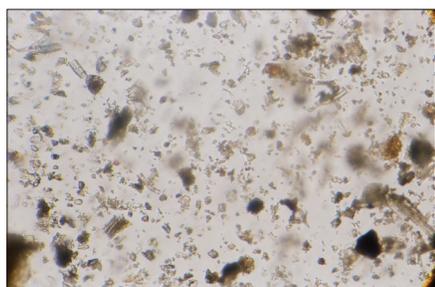


Figure 4a: Prismatic crystal of calcium oxalate



Figure 4b: Oil globules



Figure 4c: Annular vessels

Table 4: Results of TLC

Track	Visualization				R _f value
	No of spots	366 nm	No of spots	254 nm	
Track-1	5	Green	5	Green	0.13
		Bluish green		0.33	
				0.60	
				0.83	
				0.89	
Track-2	5	Green	5	Green	0.45
		Bluish green		0.53	
		Blue		0.45	
				0.53	
				0.83	

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

Table 5: Results of HPTLC

Name of Drug	Track	254 nm		366 nm	
		No of spots	R _f	No of spots	R _f
<i>Tulsi-Amla-Yashti Ghrita</i>	Unsaponifiable fraction of TAYG	5	0.26	5	0.13
			0.34		0.33
			0.45		0.60
			0.53		0.83
			0.83		0.89

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 TAYG 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



Figure 4d: Stomata

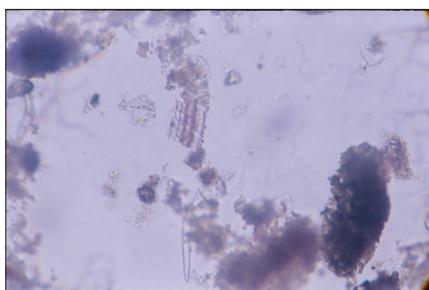


Figure 4e: Spiral vessels



Figure 4f: Lignified fibre

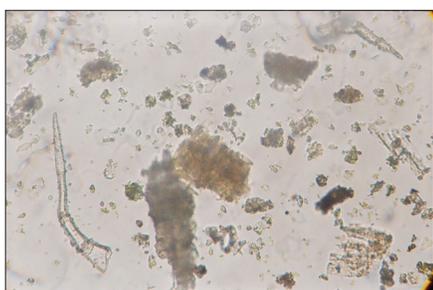


Figure 4g: Multicellular simple trichome



Figure 4h: Unicellular simple trichome

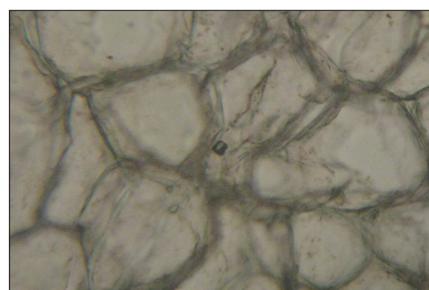


Figure 5a: Prismatic crystal

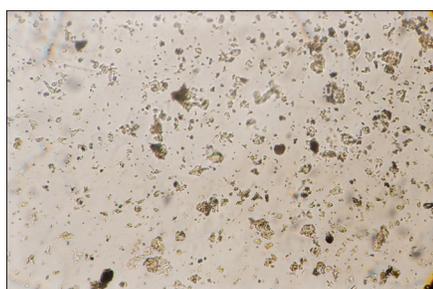


Figure 5b: Starch grains



Figure 5c: Sclereid



Figure 5d: Group of lignified fibres



Figure 6a: Cork cells

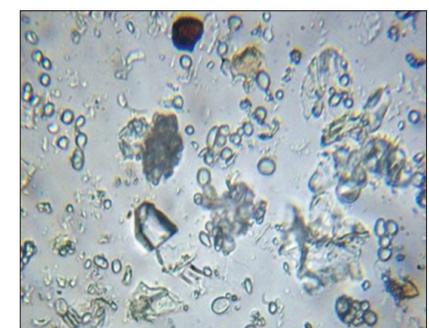


Figure 6b: Starch grains and prismatic crystals



Figure 6c: Crystal fibres

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



Figure 6d: Bordered pitted vessels



Figure 7: TLC of TAYG

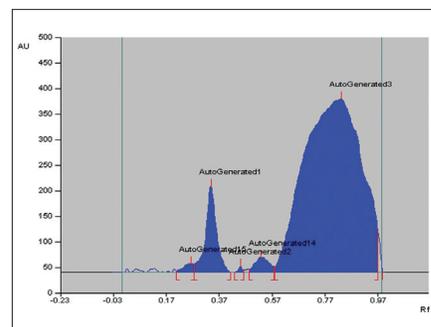


Figure 8a: HPTLC densitogram at 254nm

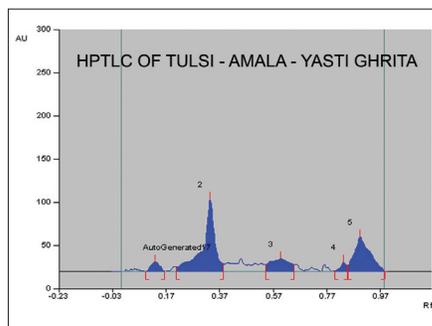


Figure 8b: HPTLC densitogram at 366nm

discussed here could be used as identifying tools for the quality assessment of *Tulasi Amla Yashti Ghrita*.

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हिन्दी सारांश

तुलसी आंवला-यष्टी घृत का औषध परिचयात्मक एवं रासायनिक परीक्षण

देबदास दत्त, हरिमोहन चन्दोला, एस्. के. अग्रवाल, विनय जे. शुक्ला, प्रीति एन. पण्ड्या

तुलसी-आंवला यष्टी घृत सिर एवं गर्दन के कैंसर में विकिरण चिकित्सा के दुष्प्रभावों को कम करने हेतु, साथ में दिया जाने वाला एक आयुर्वेदिक योग है। औषध की बेहतर कार्मुकता के लिए औषध स्वरूपात्मक अध्ययन में औषध चूर्ण का स्थूल एवं सूक्ष्मपरीक्षण किया गया जिससे औषध की गुणवत्ता एवं विशुद्धता का ज्ञान हुआ। चूर्ण के लक्षण स्वरूपात्मक अध्ययन में उसके लक्षण प्राचीन शास्त्रों एवं आदर्श निर्देशों में दी हुई मानक श्रेणी के अंतर्गत पाए गए। रासायनिक परीक्षण में निर्मित औषध का विशिष्ट भार 0.९९३० एवं pH ३.५ प्राप्त हुआ। उपयुक्त विलयन तंत्र बनाने के बाद TLC एवं HPTLC किया गया जिसमें TLC में अधिकतम ५ निशान एवं HPTLC में ९ निशान प्राप्त हुए एवं विभिन्न परिष्करण विधियों द्वारा बनाए गए नमूनों द्वारा एक समान Rf मान प्राप्त हुआ जो कि तुलसी-आंवला यष्टी घृत में कुछ विशिष्ट तत्वों की उपस्थिति को दर्शाता है एवं इन तत्वों के सरल पृथक्करण में सहायक है।