Research Article
ISSN: 0974-6943

Sunita Shailajan et al. / Journal of Pharmacy Research 2012,5(2),1112-1115

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Quality assessment of a traditional oil based Ayurvedic formulation :*Yashtimadhuka Taila*

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Received on:17-11-2011; Revised on: 21-12-2011; Accepted on:28-01-2012

ABSTRACT

Yashtimadhuka Taila (*YMT*) is an ancient Ayurvedic oil based formulation prescribed for *Palita* (graying of hair), *Keshpatana* (falling of hair) and *Smasru Patana* (falling of beard and moustache). Though *YMT* is an age old formulation, not much attempt has been made towards its standardization. The current work aims to standardize *YMT* using modern bioanalytical tools. *YMT* was prepared in house as per the classical reference and the content of gallic acid from *YMT* and its ingredients was determined using HPTLC. It was also subjected to preliminary phytochemical, physicochemical and stability evaluation. Skin irritation potential of *YMT* was tested in rabbits as a safety parameter.

Key words: *Yashtimadhuka Taila,* HPTLC, gallic acid, standardization, safety evaluation

INTRODUCTION

Yashtimadhuka Taila (*YMT*); an Ayurvedic medicated oil, is used as a remedy for hair related disorders like *Palita* (graying of hair), *Keshpatana* (falling of hair) and *Smasru Patana* (falling of beard and moustache). As per *Bhaishajyaratnavali* (a classical Ayurvedic treatise) cited in Ayurvedic Formulary of India [1], the formula composition of *YMT* includes *Tila Taila* (*TT*), *Amlaki Svarasa* (juice of fresh macerated *Amla*), *Kshira* (Cow's milk) and *Kalka* of *Yashti* **(Table 1)**. Due to the lack of proper pharmacopoeial standards, the traditional method of preparing and processing *YMT* results in its inadequate quality and inconsistency, which further results in batch to batch variation. Hence, there is a need to standardize *YMT* and prepare it using scientific methods.

Table 1: Formula composition of *YMT* as per Ayurvedic Formulary of India

Sr. No.	Ingredients Sanskrit name	Botanical identity	Quantity
1	*Tila Taila*	Oil from seeds of *Sesamum indicum* L.	8 parts
2	*Yashti*	Root of *Glycyrrhiza glabra* L.	1 part
3	*Kshira/Godugdha* (Cow's milk)	-	32 parts
4	*Amalaki Swarasa*	Pericarp of *Phyllanthus emblica* L.	32 parts

In the present work, *YMT* was prepared in the Herbal Research Laboratory, Mumbai as per the classical reference [1] using the raw materials of pharmacopoeial quality. Preliminary phytochemical evaluation of *YMT* was carried out as per the standard methods [2]. Quality of *TT* and *YMT* was assessed and compared on the basis of their respective physicochemical parameters [3].

Gallic acid **(Figure 1)**, a bioactive marker reported to have antioxidant [4], anti-inflammatory [5] and anticancer [6] properties, is among the active components found in plant ingredients used for the preparation of *YMT.*

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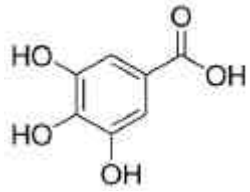

Figure 1: Structure of gallic acid

The process of extraction of phytoconstituents from *YMT* was optimized to resolve the marker compounds efficiently and to achieve a good fingerprint. A precise, accurate and reproducible HPTLC method was developed and validated for quantification of gallic acid from the complex matrix of *YMT.* Though there are reports on estimation of gallic acid using TLC from herbal formulations [7-9], there is no data reported for the estimation of gallic acid from *YMT*. Thus, this work of estimating gallic acid from *Taila* matrix (*YMT*) is first of its kind. The skin irritation potential of *YMT* was tested on rabbits as per reported methods [10, 11].2.

MATERIALS AND METHODS:

2.1 Materials:

Plant raw materials used for the preparation of *YMT* namely *Yashti* and *Amlaki* were procured from Bharat traders, Pydhonie Road, Mumbai and authenticated by Dr. Sunita Shailajan, Ramnarain Ruia College, Mumbai. *TT* was collected from the pharmacy, while *Kshira* (Amul brand) was purchased from the local market. The plant raw materials were dried in the incubator at 40º C for a week, powdered, sieved through 85-mesh (BSS) sieve and stored in air tight containers at room temperature.

2.2 Standard and reagents:

The organic solvents and chemicals of analytical grade were procured from Merck Specialties Pvt. Ltd., Mumbai. Gallic acid (> 99 % purity) was procured from Sigma Aldrich Chemical Company, Germany.

2.3 Quality evaluation of raw materials:

Powders of *Yashtimadhu* and *Amlaki* were analyzed in terms of proximate parameters like foreign matter, ash value (total ash, acid insoluble ash and water soluble ash) and loss on drying using the standard pharmacopoeial

method [3]. Adulterants like urea, milk powder, sodium bicarbonate, detergent, cane sugar, formalin etc in *Kshira* were analyzed [12] prior to preparation of *YMT.*

2.4 Preparation of Ayurvedic formulation:

2.4.1 Preparation of *Kalka* of *Yashti* and *Amlaki Swarasa*:
Kalka of *Yashti* was prepared by mixing the powder of *Yashti* in sufficient amount of water. *Amalaki Swaras* was prepared by mixing the coarse powder of *Amlaki* with water (eight times to the weight of *Amalaki*). The mixture was heated indirectly on a mild flame until the volume of water reduced to one fourth of the original volume. The mixture was then filtered through a muslin cloth and the filtrate was used as *Amlaki Swaras.*

2.4.2 Preparation of *YMT*:
YMT was prepared by heating *TT* indirectly on *Mridu Agni* (mild flame, 80-90^{0}C) followed by the addition of *Yashti Kalka* and *Amla Swaras.* This mixture was stirred intermittently till the *Kalka* became slimy. As soon as this stage was attained, the heating was stopped, *Godugdha* was added to the mixture and the mixture was kept standing overnight. Next day, the mixture was heated indirectly on a mild flame till it attained *Sneha Siddhi Lakshanas* [completion test for chief desired characteristics like *Gandha-Varna-Rasotpatti* (desired smell, colour and taste), *Shabdahinata* (no cracking sound), *Phenodgaman* (appearance of froth) and *Vartivat Kalka* (paste of herbal drugs can be rolled between fingers)]. The mixture was filtered when hot and used for further analysis.

2.5 Preliminary phytochemical evaluation:
To evaluate the presence of major phytoconstituents in *YMT,* phytochemical tests were performed for carbohydrates, alkaloids, flavonoids, tannins, essential oils, glycosides and resins as per the standard method [2].

2.6 Physicochemical evaluation:
Comparative physicochemical evaluation of *YMT* and *TT* was carried out with respect to acid value, saponification value, peroxide value, refractive index and weight per mL as per standard methods [3].

2.7 Preparation of standard solutions of gallic acid: Stock solution of gallic acid (1000.0 µg/mL) was prepared by dissolving 10.0 mg of accurately weighed standard in small amount of methanol and the volume was made to 10.0 mL in standard volumetric flask. Further aliquots (5.0 µg/mL – 60.0 µg/mL) were prepared from this stock.

2.8 Extraction of phytoconstituents:

2.8.1 From raw materials:
Yashti and *Amalaki* (1.0 g each) were extracted in methanol (10.0 mL), vortexed for 30 s and kept standing overnight. The extracts were filtered through Whatman filter paper no. 41. *Amalaki Swaras* (1.0 mL) was extracted with methanol (9.0 mL), vortexed for 30 s and kept standing overnight. Next day the upper methanolic layer was separated and used for further analysis.

2.8.2 Extraction from formulation (*YMT*):
To 50.0 mL of *YMT* and *TT*, 100.0 mL of aqueous methanol (90 %, v/v) was added in two separate stoppered conical flasks. This mixture was stirred for 1 hr using magnetic stirrer and stored in refrigerator (- 20ºC) for 2 days. After the completion of storage period, the upper methanolic layer was filtered through Whatmann filter paper no. 41 and used for HPTLC analysis.

2.9 HPTLC conditions:
Chromatographic separation was achieved on HPTLC plates precoated with silica gel 60 F_{254} (E. Merck) of 0.2 mm thickness with aluminum sheet support. Samples were spotted using CAMAG Linomat IV Automatic Sample Spotter (Camag Muttenz, Switzerland) equipped with syringe (Hamilton, 100 µL). Plates were developed in a glass twin trough chamber (CAMAG) pre-saturated with mobile phase. Scanning device used was CAMAG TLC Scanner II equipped with CATS 3 software. The experimental condition was maintained at 20 ± 2ºC. Detection of the marker compound gallic acid was possible under 254 nm using CAMAG Reprostar 3. ICH guidelines were followed for the validation of the developed HPTLC method [13].

2.10 Solvent system:
Solvent system consisting of toluene-ethyl acetate-formic acid (6: 4: 0.8, v/v/v) was used to resolve and quantify gallic acid from the matrix of *YMT* and its ingredients.

2.11 Estimation of gallic acid:
Samples (10.0 µL, filtrates obtained as per section 2.8) were applied in triplicate to a pre-coated silica gel 60 F_{254} HPTLC plate (E. Merck) with the Camag Linomat IV sample spotter. The plate was developed and analyzed as per the optimized chromatographic conditions (section 2.9)

.2.11 Method application:
The developed HPTLC method was applied to study the effect of storage on the stability of *YMT* samples at different time periods (at room temperature) in terms of gallic acid content.

2.12 Safety evaluation (skin irritation test):
Skin irritant potency of *YMT* was tested on three female healthy rabbits (New Zealand strain) obtained from college animal house after obtaining permission from Institutional Animal Ethical Committee. Approximately 24 hr before the test, fur (1 inch^2) was removed by closely clipping the dorsal right and left area of the trunk of each rabbit. *YMT* was applied on the dorsal right side of the intact epidermis of the rabbits, while dorsal left side was selected for control (without application of *YMT*). Observations for irritation in terms of erythema and oedema according to Draize Test [10, 11] were recorded at 24, 48, and 72 hours after topical application of *YMT* in order to check the occurrence of adverse effects.

3. RESULTS & DISCUSSION:
Standardization is an essential factor for ASU preparations in order to assess their quality based on the concentration of chemical or bioactive marker [14]. Modern bioanalytical techniques like HPTLC and HPLC are being used to achieve the aforesaid objectives. In the current work, an attempt has been made to standardize *YMT* using scientific methods.

The results of proximate analysis of ingredients of *YMT* for the parameters like ash values (total ash, acid insoluble ash and water soluble ash), loss on drying and foreign matter were in compliance with the limits documented in the Pharmacopoeia [results not shown,15]. In house preparation of *YMT* was carried out as per the classical reference **(Table 1)**.

Table 2: Comparative physicochemical evaluation of *YMT* and *TT*

Parameters		Results YMT	TT
Acid value (mg KOH/g)	Mean± S. D., n=3	0.5613 ± 0.0005	0.4433 ± 0.0057
Saponification value (mg KOH/g)		196.8 ± 0.0152	189.34 ± 0.0115
Peroxide value (mg KOH/g)		1.0656 ± 0.0005	7.5343 ± 0.0015
Refractive index		1.4662 ± 5.7735	1.4641 ± 5.7735
Specific gravity (g/mL)		4.3466 ± 0.0351	1.5233 ± 0.2482

The preliminary phytochemical evaluation of *YMT* revealed the presence of carbohydrates, alkaloids, tannins and essential oils whereas flavonoids and resins were absent. Increase in acid value, saponification value and specific gravity of *YMT* was observed when compared with *TT*. Peroxide value of *YMT* was reduced after processing of *TT*. There was no change observed in refractive index of *YMT.* These results suggested that the traditional method of *Taila* preparation affects physicochemical parameters of the base oil **(Table 2)**.

The HPTLC method for estimation of gallic acid was validated in terms of specificity, precision, sensitivity, recovery and ruggedness as per ICH guidelines [13]. Response for gallic acid was found to be linear in the range of 5-60 µg/mL (r^2 = 0.998) which resulted as a regression equation y = 28.82 x - 60.59. This equation was used to determine the piperine content of *YMT* and its ingredients. Developed HPTLC method was found to be precise with % RSD < 2 % for intra-day and inter-day precision. LOD and LOQ value for gallic acid was found to be 0.02 and 0.04 µg/mL respectively. The average recovery at three different levels of gallic acid for formulation was found to be 97.83 %. The method was found rugged for the parameters like change in analyst, change in mobile phase composition, change in spotting volume etc.

Among the various solvent systems tested, the mixture containing toluene-ethyl acetate-formic acid (6: 4: 0.8, v/v/v) gave the best resolution for gallic acid (R_f = 0.34) from the formulation matrix which enabled its quantification as well as phytochemical fingerprint **(Figure 2)**. The identity of the band of gallic acid in *YMT* was confirmed by comparing its UV absorption spectra with that of the standard.

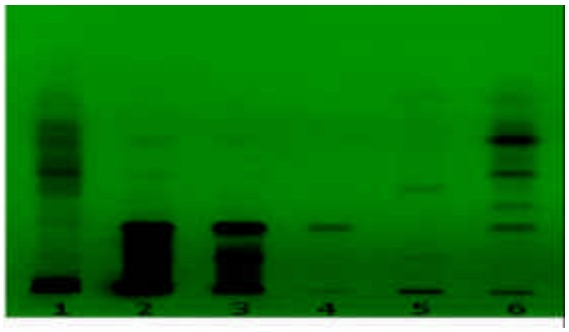

Figure 2: HPTLC Detection of gallic acid from *YMT* and its ingredients at 254 nm, Track details - 1: *Yashti*, 2: *Amalaki*, 3: *Amalaki Swaras*, 4: Gallic acid, 5: *TT*, 6: *YMT*

Table 3: Gallic acid content in *YMT* and its ingredients using HPTLC

Sample	Gallic acid content (mg/mL) [Mean ± S. D., n = 3]
Amalaki	3.618 ± 0.0131
Amalaki Swarasa	1.878 ± 0.0125
Yashtimadhuka Taila	1.026 ± 0.0085

YMT samples subjected to storage up to 1.5 months showed variation in gallic acid content. It was observed that the content of gallic acid decreased on prolonged storage **(Table 4, Figure 3)** when compared to freshly prepared formulation. The results of stability studies are supported by the frequent references in the classical Ayurvedic texts regarding the use of *Taila* [16].

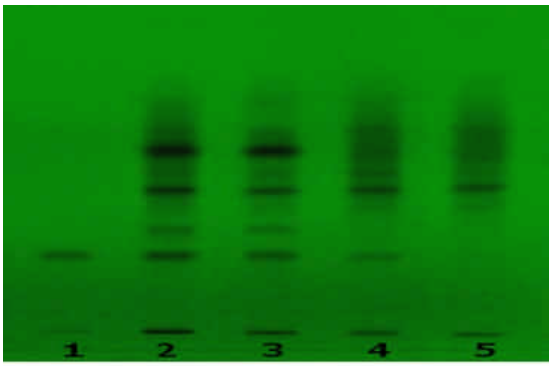

Figure 3: Stability samples of *YMT* on HPTLC at 254 nm, Track details – 1: Gallic acid, 2: 0 month sample, 3: 0.5 month sample, 4: 1.0 month sample, 5: 1.5 month sample

Table 4: Stability of *YMT* samples stored at different storage periods in terms of gallic acid content

Storage period (months)	Gallic acid content (mg/mL) [Mean ± S. D., n = 3]
0	1.026 ± 0.0085
0.5	0.890 ± 0.0087
1.0	0.580 ± 0.0105
1.5	Not detectable

Safety of the formulation was established in terms of skin irritation test which revealed that *YMT* has adequate safety margin to be used on human skin [Primary irritation index (P. I. I.) = 0; **(Table 5)**. These methods will help in establishing the quality and efficacy of the traditional formulation *YMT*.

Table 5: Skin irritation potential of *YMT* in terms of primary irritation index (P. I. I.) in rabbits

Rabbit ID	Total erythema + oedema						Average
	24 hrs		48 hrs		72 hrs		
	Control	Test	Control	Test	Control	Test	
R- 01	0	0	0	0	0	0	0
R-02	0	0	0	0	0	0	0
R-03	0	0	0	0	0	0	0
Primary irritation index (P. I. I.)							0

4. CONCLUSION:

Results of the present study can be used to characterize the samples in industry to check their uniformity. The obtained values of physical, chemi cal and biological parameters for *YMT* can be adopted to lay down new pharmacopoeial standards to be followed in its preparation with batch to batch consistency.

5. ACKNOWLEDGEMENT

We acknowledge AYUSH, Ministry of Health, Government of India under the APC Scheme for supporting this project. Authors are also thankful to Ms. Gauri Swar for her technical assistance.

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Source of support: AYUSH, Ministry of Health, Government of India, Conflict of interest: None Declared