

**Research Article****QUALITY CHARACTERIZATION OF MADHUYASHTI GHRITA****Meda Mruthyumjaya Rao¹, P. Hemant Kumar², Purnendu Panda³, Sangeeta Mukhi⁴, Anindya Bose^{5*}**^{*1}Director, Central Ayurveda Research Institute for Hepatobiliary Disorders, Bharatpur, Bhubaneswar, Odisha.² Professor, Dept. of Shalya, National Institute of Ayurveda, Jaipur, Rajasthan.³ Research Officer, Central Ayurveda Research Institute for Hepatobiliary Disorders, Bhubaneswar, Odisha.⁴ Research Scholar, ^{*5} Professor, Department of Pharmaceutical Analysis, School of Pharmaceutical Sciences, Siksha 'O' Anusandhan (Deemed to be University), Kalinga Nagar, Bhubaneswar, Odisha, India.**ABSTRACT**

Madhuyashti Ghrita, is an Ayurvedic medicated ghee preparation containing *Yashtimadhu*. It is used for treating external ulcers and wounds in vital points of the body. However, there is no quality monograph available for *Madhuyashti Ghrita*. Hence, the present work was carried out to characterize this *Ghrita* to confirm its identity, quality and purity. This work reported various pharmacognostic and physicochemical parameters of *Madhuyashti Ghrita* along with its TLC based rapid fingerprinting as per the present standards of Ayurvedic Pharmacopoeia of India. Moreover, the presence of toxic contaminants like heavy metals, and microbial load were also evaluated. In organoleptic evaluation, the *Ghrita* was found to be semi-solid and dark yellow in colour, sweet and bitter in taste with a characteristic and pleasant odour. The values of the physicochemical parameters such as acid value, saponification value, peroxide value, refractive index, rancidity and pH value of the *Ghrita* were also assed. In the microscopical analysis of the rhizomes of *Yashtimadhu*, the presence of pitted vessels, fibres, prismatic crystals and starch grains were noted as a confirmation characteristic for the genuinely of this raw material in *Madhuyashti Ghrita*. The amount of heavy metals such arsenic, lead, mercury, cadmium, nickel, zinc, copper and chromium as well as total bacterial count and total fungal count were found to be much below their API limits. The TLC showed different characteristic spots indicating the presence of *Yashtimadhu* in this product. The parameters of *Madhuyashti Ghrita* presented in this paper may be utilized for preparing a quality monograph for this product.

KEYWORDS: *Madhuyashti Ghrita*, Pharmacognostic, Physicochemical, Heavy metals, Microbial limit test.

INTRODUCTION

According to Ayurveda, *Snehakalpana* are groups of medicated preparations in which active principles are extracted in a suitable fatty media either *Taila* (oil) or *Ghrita*.^[1] *Ghritas* are a type of *Snehakalpana* containing ghee as the base to dissolve or hold the fat-soluble components of the ingredients. The active principles are extracted by boiling *Kashayas* (decoctions) and *Kalkas* (a fine paste of the drugs) in ghee to almost dehydration.^[2] *Madhuyashti Ghrita* is an Ayurvedic formulation containing

Kashaya of *Yashtimadhu* (*Glycyrrhiza glabra* Linn) dissolved in *Ghrita* or cow ghee (Table 1). This formulation is prescribed by Ayurvedic physicians for treating external ulcers and wounds in vital points of the body.^[3] To the best of our knowledge, there is no report regarding standardization of *Madhuyashti Ghrita* till date, hence the present work was carried out to characterisation of this *Ghrita* to conform its identity, quality and purity.

Table 1: Composition of *Madhuyashti Ghrita*

Sanskrit Name	Scientific Name	Part used	Quantity
<i>Yashtimadhu</i>	<i>Glycyrrhiza glabra</i> Linn.	Rhizome	50 part
<i>Ghrita</i>	-	-	200 part
Water	-	-	800 part

MATERIALS AND METHODS

Collection and Authentication of Raw Drugs

The ingredients of *Madhuyashti Ghrita* were purchased from the herbal shops of Cuttack (India). They were authenticated at Central Ayurveda Research Institute for Hepatobiliary Disorders, Bharatpur, Bhubaneswar, India. The quality of the liquorice was found to of desired standard.^[4]

Method of Preparation of *Madhuyashti Ghrita*

The *Madhuyashti Ghrita* was prepared according to the method of Ayurvedic Pharmacopoea of India (API). In brief, one part of *Yashtimadhu churna* (powder) was added with sixteen parts of water and boiled till the volume of water reduced to four parts. The prepared decoction was filtered and the filtrate was added with four parts of cow ghee. The mixture was heated till evaporation of its water content.^[5]

Pharmacognostic Characterization

Organoleptic Study

The prepared *Madhuyashti Ghrita* and its two ingredients, i.e., *Yashtimadhu* powder and Cow ghee were subjected for evaluation of different sensory characters like colour, taste odour, etc., and the observations were documented.^[6]

Powder microscopy

The *Yashtimadhu churna* was passed through 80 mesh sieve, washed with plain water. Small amount of samples were treated separately with different chemicals like iodine, chloral hydrate, pholorglucinol or potassium iodide. Thereafter a drop of glycerine was added in each mixture for mounting in a microscopic slide. The sample characteristics were examined using a camera fitted Carl Zeiss binocular microscope.^[7]

Physico-chemical study

Various important physicochemical parameters like Acid value, saponification value, Refractive Index, peroxide value, viscosity, Ester value, rancidity and pH were estimated for the *Madhuyashti Ghrita* as per standard protocols.^[8]

Assessment of Toxicity

Heavy Metal Limits

Heavy metals were quantified by atomic absorption spectroscopy (AAS) using the PERKIN ELMER AAS-200 instrument. The metals like Lead (Pb), Cadmium (Cd), Copper (Cu), Zinc (Zn), Nickel (Ni) and Chromium (Cr) in the sample were dissolved by multi-acid digestion and the filtrate was subjected to AAS.^[9] However the volatile metals like Mercury (Hg) and Arsenic (As) were digested using Nitric acid-Hydrochloric Acid- Potassium Permanganate solvent system and analysed through Mercury

Vapour Atomization (MVA) and Hybrid Vopour Generation (HVG) system respectively.^[9] The tested metals were quantified using the respective calibration curves for these metal standards.

Microbial Limit Test

The microbial load in the prepared *Madhuyashti Ghrita* was analysed according to the specified protocol of API.^[10] The parameters included total bacterial count and total fungal count along with freedom from four indicator pathogenic bacteria such as *E.coli*, *Salmonella*, *P.aeruginosa*, as well as *S.aureus*.

Thin Layer Chromatography (TLC)

Sample Preparation: *Madhuyashti Ghrita* (5gm) is partitioned between 10ml each of hexane and methanol in a separating funnel to dissolve the fatty matter in hexane. The hexane layer was discarded and the collected methanolic fraction was washed repeatedly in hexane till it was free from fat. The methanolic fraction was filtered through 0.22 μ filter paper and the filtrate was concentrated upto 5ml for application in TLC plate. On the other hand, accurately weighed 1gm of *Yashtimadhu* powder was dissolved in 20ml methanol, filtered and evaporated up to 5ml for TLC spotting.

Solvent System: Toluene: Ethyl Acetate: n-hexane (7.3:2.5:0.2 v/v) showing good spot resolution was selected for TLC analysis.

Spotting: *Madhuyashti Ghrita* and *Yashtimadhu* samples were applied on 0.2mm precoated Silica Gel 60 F₂₅₄ plates (Merck KGaA) for development.

Visualization: The developed TLC plates were visualized at both short wave (254nm) and long wave (366nm) UV light.^[11]

RESULTS AND DISCUSSION

Although *Madhuyashti Ghrita* is a popular Ayurvedic formulation, presently there is lack of pharmacopoeial standards or any other quality monographs laid down for quality control of this product. *Madhuyashti Ghrita* available in market therefore may not have the desired quality and batch-to-batch consistency. Looking at the need for development of characterisation parameters for *Madhuyashti Ghrita*, the present work reports its pharmacognostic parameters and toxicity assessment.

Every parameter reported here for the *Madhuyashti Ghrita* has significant contribution in its identity, quality and safety. For example, foreign matters are specified in pharmacopoeias as adulteration due to presence of useless parts of the same or other plants, yeasts and moulds, insects and their excreta, and chemical residues etc., which are very common and can be harmful. Conformation with organoleptic characteristics is considered as part of

acceptance and identification criteria of herbal drugs. Powder microscopy is very useful diagnostic tool for identification of adulterants. On the other hand, the established physicochemical standards of herbal products facilitate the evaluation of consistency and quality in their routine industrial production. Presence of micro organism may be detrimental to an herbal drug as it not only reduce the therapeutic activity of the products but can also produce adverse effect to the patients. The regulatory authorities therefore prescribe the maximum acceptable limit of bacteria and fungus in the herbal products to assure their quality, safety and efficacy. The presence of heavy metals beyond acceptance limit can cause serious health problems because of their toxicity.^[12,13] TLC is an important tool in identifying and quality assurance of an herbal drug moreover it can confirm the presence or absence of individual ingredients in a multi- ingredient herbal formulation.^[14]

Considering the importance of the above mentioned parameters in quality control of herbal formulations, the classical Ayurvedic formulation *Madhuyashti Ghrita* was characterized by relevant pharmacognostic and physicochemical parameters as well as toxicity assessments by heavy metal estimation and microbial limit tests. Moreover TLC based rapid fingerprint of the *Ghrita* was also developed as per the present standards of API.

Pharmacognostic Parameters

In organoleptic evaluation, the *Ghrita* was found to be semisolid and oily in form (*Sparsha*), dark yellow in colour (*Rupa*), sweet and bitter in taste (*Rasa*), with a characteristic and pleasant odour (*Gandha*) as mentioned in Table 2. The values of the physicochemical parameters such as acid value, saponification value, peroxide value, refractive index, rancidity and pH value of the *Madhuyashti Ghrita* were assed and presented in Table 3.

Table 2: Organoleptic Properties of Raw Materials and *Madhuyashti Ghrita*

Test	Cow's ghee	<i>Yashtimadhu</i>	<i>Madhuyashti Ghrita</i>
Colour	Golden yellow	Brownish yellow	Dark yellow
Odour	Characteristic, pleasant	Sweetish	Characteristic, pleasant
Texture	Granular, oily	-	Oily
Touch	Oily	-	Oily
Taste	Characteristic	Sweet, bitter	Sweet, bitter

Table 3: Physicochemical Parameters of *Madhuyashti Ghrita*

Test	Result
Acid value	4.712
Saponification value	214.79
Peroxide value	8.12
Refractive Index	1.4518
Rancidity test	Negative
pH value	5.2

In the microscopical analysis of the rhizomes of *Yashtimadhu*, the presence of pitted vessels, fibres, prismatic crystals and starch grains were noted as a confirmation characteristic for the genuinely of this raw material in *Madhuyashti Ghrita* (Fig 1).

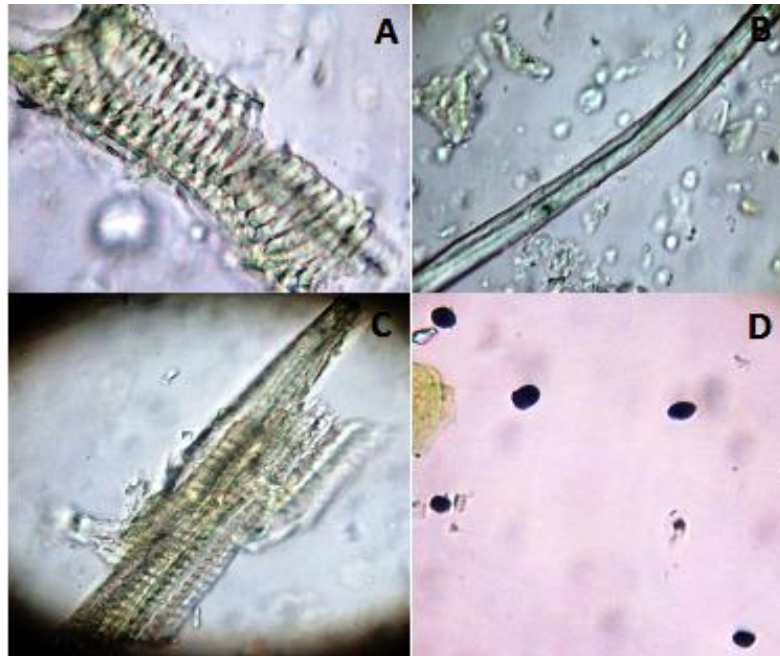


Fig 1: Photographs of powder microscopy of *Madhuyashti Ghrita*. (A) Pitted vessels of *Yashtimadhu*, (B) Fibres of *Yashtimadhu*, (C) Prismatic crystals of *Yashtimadhu*, (D) Starch grains of *Yashtimadhu*

Determination of Toxic Contaminants

The amount of heavy metals such arsenic, lead, mercury, cadmium, nickel, zinc, copper and chromium were detected within the API permissible limits for *Madhuyashti Ghrita* and the results are shown in Table 4. The microbial load of the *Madhuyashti Ghrita* was determined as per the guidelines of API. Total bacterial count and total fungal count (yeast and mould) was respectively found to be 212 CFU/mL and 2 CFU/mL, which were much below their API limits. Moreover, the product was found to be free from the four API specified indicator bacterial species namely *E. coli*, *Salmonella*, *S. aureus* and *P. areuginosa* (Table 5, Fig2).

Table 4: Heavy Metal Content in *Madhuyashti Ghrita*

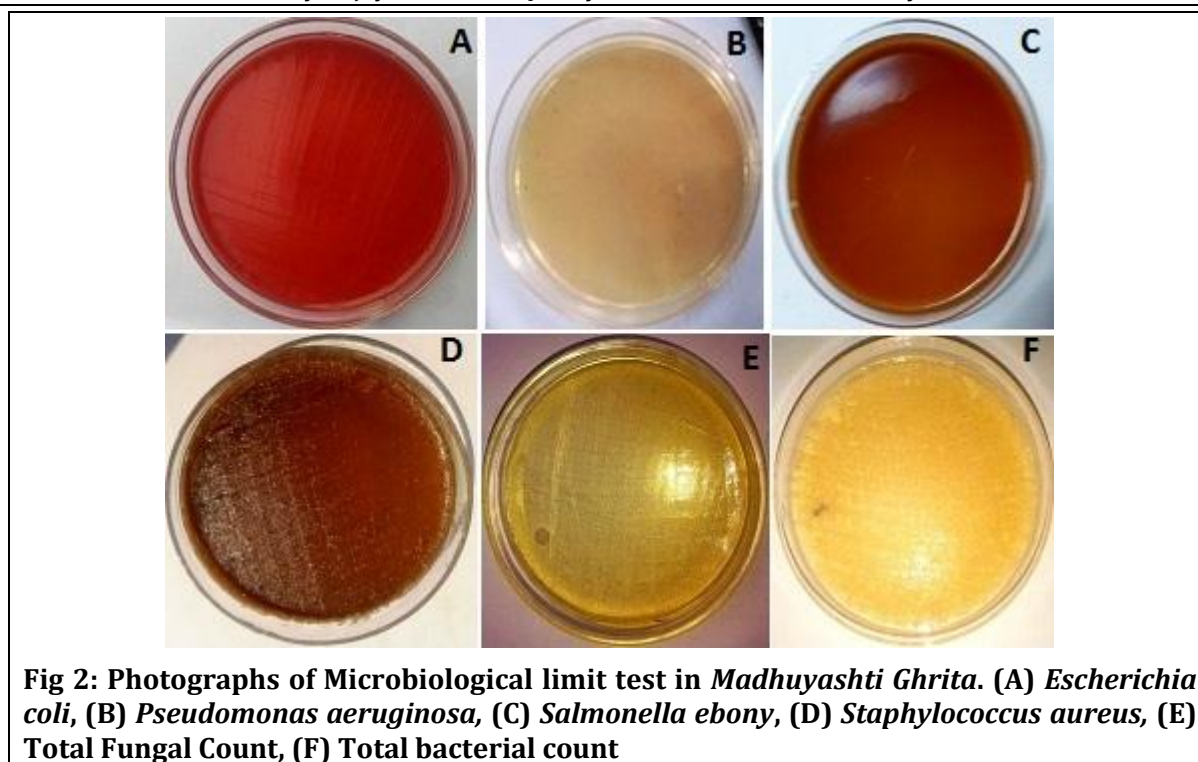
Metal	Amount found (ppm)	Standard API limit (ppm)
Arsenic	0.55	3
Lead	3.60	10
Mercury	1.17	1
Cadmium	0.04	0.3
Nickel	1.30	NS
Zinc	4.60	NS
Copper	1.02	NS
Chromium	1.65	NS

*NS -Not specified in API

Table 5: Microbial Load in *Madhuyashti Ghrita*

Microbial Analysis	Observation	API Limit
Total bacterial count	212 CFU/mL	NMT 10 ⁵ CFU/ml
Total fungal count	2 CFU/mL	NMT 10 ³ CFU/ml
<i>Escherichia coli</i>	NF	To be absent
<i>Salmonella spp.</i>	NF	To be absent
<i>Staphylococcus aureus</i>	NF	To be absent
<i>Pseudomonas areuginosa</i>	NF	To be absent

*NF -Not found



TLC study

The results of the TLC study of the methanolic extracts of the *Madhuyashti Ghrita* in the solvent system of Toluene: Ethyl Acetate: n-hexane (7.3:2.5:0.2 v/v) are shown in Table 6 and the plate images shown in Fig 3 and Fig 4. The TLC plates visualized at 254nm and 366nm showed different characteristic spots indicating presence of *Yashtimadhu* in the product.

Table 6: Comparative TLC Analysis of *Yashtimadhu* vs. *Madhuyashti Ghrita* at wavelength of 254 and 366nm

R _f Values			
254 nm		366 nm	
Track A (<i>Yashtimadhu</i>)	Track B (<i>Madhuyashti Ghrita</i>)	Track A (<i>Yashtimadhu</i>)	Track B (<i>Madhuyashti Ghrita</i>)
0.2	0.2	0.2	0.2
0.4	0.4	0.4	0.4
0.8	0.8	0.8	0.8
0.12	0.12	0.12	0.12
0.16	0.16	0.16	0.16
0.21	0.21	0.21	0.21
0.28	0.28	0.28	0.28
0.36	0.32	0.32	0.33
0.4	0.36	0.36	0.36
0.46	0.4	0.4	0.4
-	0.46	0.46	0.46
-	-	0.5	0.5

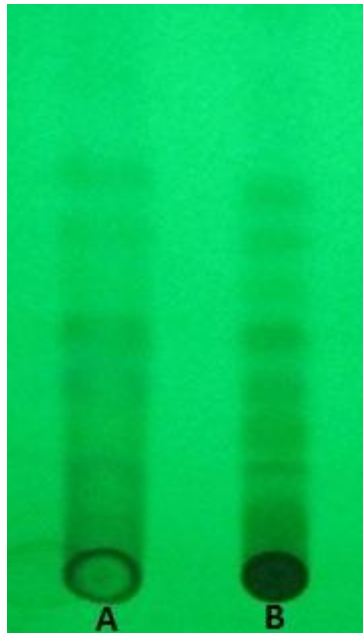


Fig 3: Photographs of TLC plates of *Madhuyashti Ghrita* and *Yashtimadhu* at 254nm- (A) *Madhuyashti Ghrita* (B) *Yashtimadhu*

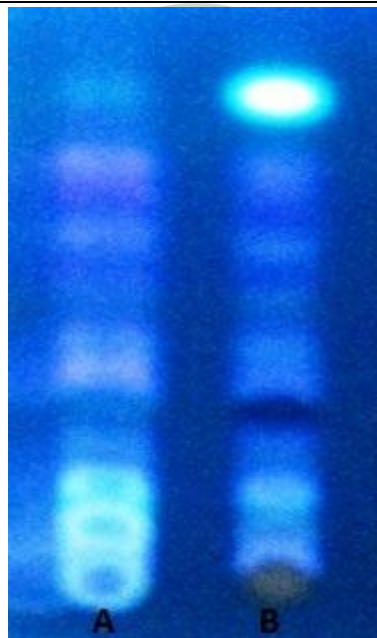


Fig 4: Photographs of TLC plates of *Madhuyashti Ghrita* and *Yashtimadhu* 366nm before derivatization- (A) *Madhuyashti Ghrita* (B) *Yashtimadhu*

CONCLUSION

Our study results can be utilised for development of a quality monograph for *Madhuyashti Ghrita*. The methodologies adopted in assessment of quality and safety of the *Madhuyashti Ghrita* may serve as reference for development of standard operating procedures to be adopted for quality control assurance of different Ayurvedic *Ghrita* formulations.

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