IJBPAS, February, 2024, 13(2): 616-624

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PHYSICOCHEMICAL ANALYSIS AND HPTLC FINGERPRINTING OF *BALAGUDUCHYADI TAILA* - AN AYURVEDIC POLYHERBAL FORMULATION

HIREMATH KV[1], KRISHIKA M[2*], BHUMIKA S[3], KUPATI VB[4], SHETTI PP[5] AND GUDASI SP[6]

1: Reader, Department of Kayachikitsa, KAHER's Shri BMK Ayurveda Mahavidyalaya, Belagavi, Karnataka, India

2: Department of Rasayana evam Vajeekarana, KAHER's Shri BMK Ayurveda Mahavidyalaya, Belagavi, Karnataka, India

3: Department of Rasashastra and Bhaishajyakalpana, KAHER's Shri BMK Ayurveda Mahavidyalaya, Belagavi, Karnataka, India

4: Assistant professor, Department of Rasashastra and Bhaishajyakalpana, KAHER's Shri BMK Ayurveda Mahavidyalaya, Belagavi, Karnataka, India

5: Research Associate (grade II), Dr. Prabhakar Kore Basic Science Research Centre, Belagavi, Karnataka, India

6: PG scholar, Department of Pharmacognosy, KLE College of Pharmacy, Belagavi, Karnataka, India

***Corresponding Author: Dr. Krishika M: E Mail: dr.ayurvedha@gmail.com**

Received 4th March 2023; Revised 25th March 2023; Accepted 6th June 2023; Available online 1st Feb. 2024

https://doi.org/10.31032/IJBPAS/2024/13.2.7758

ABSTRACT

Introduction: Ayurveda uses a variety of polyherbal formulations. *Balaguduchyadi taila* (BGT), a sesame oil based formulation is mentioned as two versions in Ayurveda. Apart from the market available BGT, the second seems to be simple with less number of ingredients. Making use of such formulations saves time, energy and resources greatly alongside therapeutic scope. An attempt is made to analyze analytical parameters of BGT as there are no standards available in Ayurvedic pharmacopoeia for this version. Doing so the genuinity and quality of such formulations can be assessed which could indirectly help to provide the best of treatments.

Methodology: After authentication and preliminary tests of raw drugs, BGT was prepared as per the standard method of preparation mentioned. BGT was analyzed for a series of analyses along with HPTLC fingerprinting.

Results: The moisture content, acid value, iodine value, saponification value, refractive index, weight per ml, and specific gravity were found to be 0.0926%, 3.349, 84.27, 187.18, 1.468, 0.919 and 0.917 respectively. HPTLC fingerprinting of methanolic extract of BGT run in toluene: ethyl acetate: n-hexane (6:3:1 v/v) showed an average R_f of 0.838 ∓ 0.039 from all the tracks. Three peaks corresponding to R_f 0.173, 0.744 and 0.853 were found in the last track.

Discussion and conclusion: Obtained analytical results are within the specified limits for sesame based oil. Average R_f corresponds to *Bala* thus indicating its presence in BGT. The above findings comply with its use clinically. Analysis of similar formulations is essential to extend Ayurvedic practice largely.

Keywords: *Bala*, *sneha kalpana*, HPTLC, fingerprinting, ayurvedic oil

INTRODUCTION:

Pharmaceutics in Ayurveda deals with a variety of dosage forms like *kashaya* (decoction), *churna* (powder), *vati* (tablet), *asava* (self-generated alcoholic preparations) etc. The dosage form is formulated in such a way to exert the greatest effect of any drug used in the formulation against specific disease and its target at the same time being conducive to the patient to whom it is prescribed. *Sneha kalpana* (fat-based preparations) is one such dosage form to use either ghee or oil, in general, as the medium to extract drug's phytoconstituents.

Baladuchyadi taila (BGT) is one such classical preparation mentioned in Sahasrayoga - a classical compendium of ayurvedic formulations, with sesame oil as the base to extract the phytoconstituents present in herbal drugs **[1]**. Commonly, the market available BGT, is extensively used in clinical practice in treating inflammatory conditions such as Vasculitis (*vatarakta*), Gouty arthritis (*gambhira vatarakta*), etc and also as a medicine for oleation before *Shodhana karma* (purificatory procedures) **[2, 3, 4]**. There is another mention of BGT from the same text that looks quite simple with the ingredients in terms of availability **[5]**. It has the drugs such as *Bala* (*Sida cordifolia* Linn), *Guduchi (Tinospora cordifolia* Linn*), Yastimadhu* (*Glycyrrhiza glabra* Linn), *Ushira* (*Vetiveria zizanoides* linn), *Musta* (*Cyperus rotundus* linn), *Rakta Chandana* (*Pterocarpus santalinus* Linn), *Go Ksheera* (cow's milk) and *tila taila* (sesame oil). Making use of such formulations with less number and easily available ingredients pave the way to

achieve greater therapeutic effects in a short time.

Analysis of quality control parameters with respect to ayurvedic formulations is as important as prescribing the same to patients as it reveals whether the formulation is fit enough to exert the desired therapeutic effect or not. Also since this formulation contains no quality standards available from Ayurvedic Pharmacopoeia of India, an attempt is made to assess the quality control parameters by contributing analytical data as a first step towards making use of this version of BGT extensively into clinical practice.

MATERIALS AND METHODS:

The raw drugs were procured and BGT was prepared in GMP-certified KLE Ayurveda pharmacy, Belagavi. Authentication of all the raw drugs was done at Central Research Facility, AYUSH – approved Drug Testing Laboratory for ASU drugs, KAHER's Shri BMK Ayurveda Mahavidyalaya, Belagavi. *Kashaya* (decoction) for BGT was prepared by adding one kg of each *Bala* and *Guduchi* to 16 liters of water and was soaked overnight and next day it was boiled and reduced to 1/4th (4 liters) of the entire quantity taken and the kashaya is kept aside.The entire process is carried out by maintaining a temperature ranging from 104-106°C by keeping the vessel open. After cooling down the *Kashaya* prepared was strained using a clean cotton cloth and kept separately. 125 g of powdered drugs including *Chandana, Ushira, Musta* and *Yashtimadhu* were mixed to the required quantity of water and *kalka* (paste) was prepared. Vessel was taken, kept on fire, upon which 2 liters of *Murchita tila taila* (processed sesame oil) was added and heated. To that prepared *kashaya* and cow's milk (4 liters) were added and boiled. Prepared *kalka* was also added and the mixture was boiled on low flame till the *siddha lakshanas* (signs of complete processing of oil) were observed. Then it was filtered through a double-layered cloth and the prepared *taila* was allowed to self-cool to room temperature.

The physicochemical analysis of the raw drugs and the BGT was done in Central Research Facility, AYUSH – Approved Drug Testing Laboratory for ASU drugs, KAHER's Shri BMK Ayurveda Mahavidyalaya, Belagavi. All the tests for the ingredients were done as per the pharmacopeial standard operating procedures mentioned in Ayurvedic Pharmacopoeia of India.

HPTLC fingerprinting was done based on the review of relevant data as there are no standards mentioned in Pharmacopoeia. Fingerprinting analysis for BGT was done in Dr.Prabhakar Kore's Basic Science Research centre, Belagavi, Karnataka. The specification for HPTLC is as follows:

Sample preparation: 5 g of BGT was added to 100 ml methanol and extracted by keeping it in an orbital shaker incubator continuously for six hours at 40℃. Later the mixture was filtered and concentrated. 35 mg of the concentrated sample was dissolved in one ml of methanol to be the sample solution of HPTLC analysis.

Instrument: CAMAG HPTLC system, consisting of Linomat 5 spotting device and TLC scanner 4 with vision CATS

Absorbent: Merck, HPTLC Silica gel 60 F_{254} (20 cm X 10 cm), thickness – 8mm,
No of tracks-15,
band length - 8mm

Solvent system: Toluene: ethyl acetate : n-hexane (6 : 3 : 1)

Solvent running distance: 70 mm

Scanning wavelength and measurement mode: 254 nm at absorbance and 371 nm at fluorescence in single **λ** and 254 to 450 nm at absorbance in the spectrum

Scanning speed: 20 mm/s in single **λ** and 20 nm/s in spectrum

Procedure: The **s**ample solution was applied in different concentrations as mentioned with a preset applicator. The solvent system in a specific ratio was taken in a twin trough chamber saturated 20 minutes prior to the commencement of chromatography. Plates were dried and loaded in the chambers and were allowed to travel upto a specified distance in the solvent. Later dried plates were visualized under the CAMAG UV cabinet and scanned for peaks.

RESULTS:

Table 1: Results of analysis of raw drugs used in BGT

Drug		Macroscopic features				Physicochemical Standards				
		Part	Colour	Odour	Taste	Foreign matter	Ash value	Acid insoluble ash	Water soluble extractive	Alcohol soluble extractive
1. *Bala*	Result	Root	Brownish	Not specific	Not specific	Nil	6.185 %	1.237 %	6.461%	3.59%
2. *Guduchi*	API	Stem	Light Brown	Not done	Bitter	<2%	<16%	<3%	>11%	>3%
	Result	Stem	Light Brown	Not done	Bitter	Nil	8.353 %	4.418 %	12.13%	4.314%
3. *Yastimadhu*	API	Stem and root	Yellowish brown or dark brown	Sweetish	Faint and Characteristic	Nil	<10%	<2.5%	>20%	>10%
	Result	Root	Yellowish brown or dark brown	Sweetish	Faint and Characteristic	Nil	5.218 %	1.341 %	39.150 %	12.151%
4. *Musta*	API	Rhizome	Dark brown or black externally, creamish yellow internally	Pleasant	Not available	<2%	<8%	<4%	>11%	>5%
	Result	Rhizome	Dark brown or black externally,	Pleasant	Not done	Nil	6.769 %	1.343 %	13.356 %	7.933%

			creamish yellow internally							
5. *Rakta Chandana*	API	Heart wood	Blood red to dark purplish red or black	Slightly astringent	Odourless	<2%	<2%	<0.3%	>1%	>3%
	Result	Heart wood	Blood red to dark purplish red or black	Slightly astringent	Odourless	Nil	1.280 %	0.269 %	2.367%	4.698%
6. *Ushira*	API	Root	Grey or light yellow to brown	Slightly bitter	Strong Aromatic	<2%	<9%	<6%	>5%	>4%
	Result	Root	Grey or light yellow to brown	Slightly bitter	Strong Aromatic	Nil	8.35%	5.95%	19.043 %	5.011%

Table 2: Results of analysis done for oil at different stages

Tests	TT		MTT	BGT
	API	Obtained result		
Colour	Light golden	Light golden	Golden yellow	Dark Reddish Brown
Odour	Pleasant	Pleasant	Pleasant	Pleasant
Form	Oil	Oil	Oil	Taila
Moisture content	---	---	0.398%	0.0926%
Refractive index at 40°C	1.4650 to 1.4665	1.4664	1.4664	1.468
Iodine value	103 to 116	107.88	98.98	84.27
Saponification value	188 to 195	192.74	210.26	187.18
Acid value	<2	1.993	2.68	3.349
Specific gravity	0.9160 to 0.9190	0.9171	0.920	0.917
Weight per ml	0.916 to 0.921	0.919	1.146	0.919

TT - *Tila taila* (sesame oil); MTT - *Murchita tila taila* (processed sesame oil)

Table 3: Microbial test results of BGT

Test for specified Microorganism (Qualitative)		
Organism	Limits (As per IP)	Results
E-coli	Absent/ 100ml	Absent
S aureus	Absent/ 100ml	Absent
P aeruginosa	Absent/ 100ml	Absent
S abony	Absent/ 100ml	Absent
Microbial limit test (Quantitative)		
Organism	Limits (As per IP)	Results
Total Bacterial count	30-300 cfc/ml	07cfc/ml
Total Fungal count	10- 100 cfc/ml	04cfc/ml

Table 4: R_f values of samples applied on different tracks

Track no	Concentration (µl)	Rf obtained
1	0.2	0.340, 0.839
2	0.2	0.076, 0.831
3	0.4	0.824
4	0.6	0.165
5	0.8	0.875
6	1	0.832
7	1.2	0.835
8	1.4	0.837, 0.924
9	1.6	0.839, 0.916
10	1.8	0.171, 0.924
11	2	0.171, 0.845, 0.926
12	2.2	0.171, 0.842, 0.924
13	2.4	0.837, 0.924
14	2.6	0.171, 0.837, 0.932
15	2.6	0.173, 0.744, 0.853
Mean R_f 0.838 ∓ 0.039		

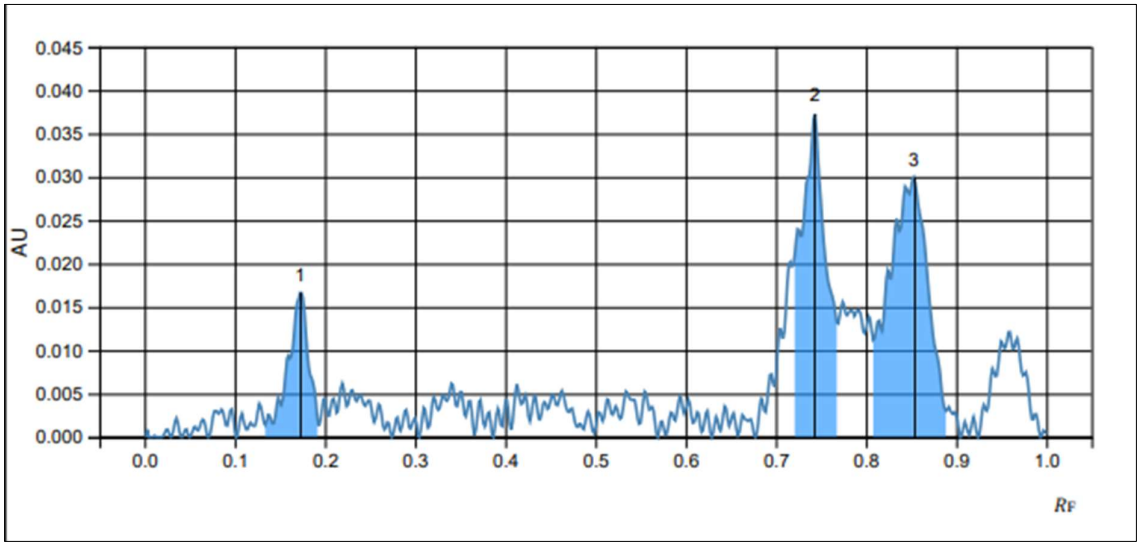

Image 01: Peaks of BGT (track 15)

DISCUSSION:

The entire preparation of BGT was done using the standard ratio of sneha kalpana. Both the hydrophilic and lipophilic layers present in the oil in water phase of emulsion in milk creates a convenient surface to extract herbal components that are hydrophilic and lipophilic in nature respectively **[6]**. The use of milk as a solvent also helps to extract the herbal drugs' nucleic acid present in DNA. It may be due to this reason that milk was selected as the choice of solvent for this formulation to extract drugs in a controlled temperature **[7]**. *Murchita tila taila* (MTT) is an intermediate product obtained after a specific process called *taila murchana* (sesame oil processing) in this preparation of BGT. It was also prepared using the standard method of preparation as mentioned in the classics **[8]**. All the macroscopic features of the raw drugs taken for the preparation are within the pharmacopeial limits indicating the genuineness and authenticity of the ingredients used for the formulation. The total ash value represents the inorganic content present in the drug after removing the organic and water part by heating method. In the entire preparation, *Guduchi* and *Ushira* have more total ash value in comparison with other drugs. Contents in the form of silica and silicates that are left as a residue after dissolving in acid media signifies the value of acid-insoluble ash. All ingredients of this formulation contains a value of at least more than 10% of acid insoluble ash indicating presence of less amounts of silica and other silica-like particles. Based on the solubility of a drug's component in different solvents, the extraneous components can be removed from certain cell wall membranes. Those are

known as extractives. Among the various solvent used for extraction water and ethanol have been reported for effectively removing the extractable components from the crude drug **[9]**. Hence for the majority of the herbal drugs water and alcohol are used as the media to find out the extractive values. Subsequent color change of plain sesame oil from light golden to golden yellow after *taila murchana* and to dark reddish brown after final preparation of BGT is indicative of infusion of herbal components into the fatty media and hence the color change is witnessed. It can be seen that moisture content is reduced in the final product BGT in spite of being exposed to the decoction of *bala* and *guduchi* through boiling after *taila murchana*. This shows the complete removal of the aqueous part while processing the medicated oil. The refractive index has no remarkable change in the complete processing of BGT. There is a remarkable decrease seen in the iodine value of plain sesame oil, MTT, and BGT. This may be indicative of the conversion of unsaturated fat molecules into saturated ones through the process of *taila murchana* and *taila paka* (boiling process of oil). This conversion indirectly increases the stability of the final product as unsaturated fats are more prone to oxidation **[10]**. The saponification value of BGT can be seen as lower than plain sesame oil. Reduced saponification values indicate the presence of more long-chain fatty acids **[11]**. This can possibly prevent the risk of developing hypertriglyceridemia on the consumption of this formulation internally for any of its indications specified **[12]**. **The a**cid value seems to be increasing in all the samples of oils tested. Hydrolysis of triglycerides into fatty acids is becoming evident with this value. The average R_f value of all 15 tracks is 0.838 ∓ 0.039. In the HPTLC analysis of *Sida* species done by Sayyada Khatoon et.al, similar R_f values were found to be present in *Sida cordifolia* **[13]**. By this, we may justify that the preparation method of BGT has actively imbibed the major phytoconstituent of *Sida cordifolia*. Tracks 10, 11,12,14 and 15 show a similar R_f of 0.17. This can probably represent one of the R_f of *Guduchi* as reported by Usmani Khushboo in her work **[14]**. Another classical oil preparation of Ayurveda such as *Dhanwantaram tailam* and the market available BGT too shares a few ingredients from the study drug BGT such as *Bala, guduchi, chandana* and *yashtimadhu* **[15, 16]**. It is quite important to note that the R_f obtained from TLC of the same oils run in the same extract and mobile phase in pharmacopeial standards were found to be coinciding with those results obtained here. The R_f around 0.3, 0.7, 0.8, and 0.9 are exhibited in all three formulations. These values could probably correspond to the presence of mutually

inclusive drugs such as *bala, guduchi, yashtimadhu* and *rakta chandana.*

CONCLUSION:

Ayurveda describes infinite formulations in its vast ocean of classics. Each formulation is one of its kind, right from the contents it possesses to the indications it is specified for. BGT in this study with fewer easily available ingredients is a formulation with a greater scope for research in both clinical and preclinical aspects. The analysis and fingerprinting done in this work are characteristic of this formulation that would help in testing the quality when prepared. On that note, selecting such a formulation with no analytical data available to work on leaves behind new horizons to discover and analyze various Ayurvedic pharmaceutical procedures.

ACKNOWLEDGMENT: Nil

REFERENCES:

[1] Nishteshwar K, Vidyanath R (Editor), Saharayogam, Taila Prakara, Varanasi, Chowkhambh Sanskrit Series Office, 2017,Verse 11, 116.

[2] Gururaja D, Hegde V, Ayurvedic Management Of Systemic Lupus Erythematosus Overlap Vasculitis, Journal Of Ayurveda And Integrative Medicine, 2019,*10*(4), 294-298.

[3] Mooss Ae, Smina Pb, Nair Pk, Traditional Ashtavaidyan Ayurvedic Therapy In The Functional Improvement Of Patients With Gouty Arthritis, Journal Of Ayurveda Physicians & Surgeons (Japs)(Eissn 2394-6350), 2019 Apr 12,6(1),7-10.

[4] Nair As, Jose D, Shukkoor Ma. Upper Extremity Arterial Occlusive Disease Managed Through Leech Application And Adjuvant Ayurveda Medicines–A Case Report. International Journal Of Ayush Case Reports. 2022 Mar 13,6(1),1-6.

[5] Department Of Indian System Of Medicine, The Ayurvedic Pharmacopeia Of India. Part -1, Second Edition, Govt Of India, New Delhi, 2003, 8(416).

[6] Sharma A, Extraction Of Medicinal Principles With Milk, Ancient Science Of Life, 1994, 13, 239-41.

[7] Verma, S.K, Biswas N, A Novel Nucleic Acid Extraction Method From Aromatic Herbs And Dried Herbal Powders Using Cow Skim Milk, *Sci Rep* **10**,11513 (2020), Https://Doi.Org/10.1038/S41598-020-68467-4.

[8] Kaviraj Shri Govindadassen, Bhaishajya Ratnavali, Edited With Siddiprada Hindi Commentary, By Prof. Siddhinandan Mishra, Varanasi, Published By Chaukhamba Surbharti Prakashan,

Reprint 2015, 5th Chapter, 1264 Verse, 206.

[9] Celignis Analytical, Chemistry Of Biomass, Background And Relevance To Valorisation, https://Www.Celignis.Com/Chemistry.Php#Extractives

[10] Unyime Ebong, Christopher, A Comparison Of Iodine Values Of Some Co. 2019, 10.13140/Rg.2.2.24160.02560.

[11] Ivanova M, Hanganu A, Dumitriu R, Tociu M, Ivanov G, Stavarache C, Popescu L, Ghendov-Mosanu A, Sturza R, Deleanu C, Chira Na, Saponification Value Of Fats And Oils As Determined From 1h-Nmr Data, The Case Of Dairy Fats, Foods, 2022 May 18,11(10), 1466. Doi: 10.3390/Foods11101466. Pmid: 35627035; Pmcid: Pmc9140812.

[12] Rozenn N. Lemaitre, Barbara Mcknight, Nona Sotoodehnia, Amanda M. Fretts, Waqas T, Qureshi, Xiaoling Song, Irena B. King, Colleen M. Sitlani, David S. Siscovick, Bruce M, Psaty, Dariush Mozaffarian, Circulating Very Long-Chain Saturated Fatty Acids And Heart Failure, The Cardiovascular Health Study. Journal of the American Heart Association, 2018, 7 (21).

[13] Khatoon S, Srivastava M, Rawat A, Mehrotra S, Hptlc Method For Chemical Standardization Of Sida Species And Estimation Of The Alkaloid Ephedrine, Jpc-Journal Of Planar Chromatography-Modern Tlc, 2005 Oct 1,18(105),364-7.

[14] Khushboo Usmani.Hptlc Fingerprint Profile Of Stems Of Tinospora Cordifolia (Wild) Miers And Roots Of Hemidesmus Indicus (L.) R.Br With Their Polyherbal Mixture, 2020 Https://Doi.Org/10.5281/Zenodo.4010240

[15] Ayurvedic Formulary Of India, Government Of India, Ministry Of Health And Family Welfare, The Controller Of Publications, Civil Lines, Delhi- 110054 Part 1, 2nd Ed., Department Of Ayush ,2007 137.

[16] Ayurvedic Pharmacoepia Of India. Government Of India, Ministry Of Health And Family Welfare, The Controller Of Publications, Civil Lines, Delhi- 110054 Part 2 Vol 1. 1st Ed., Department of Ayush ,2007 119.