RESEARCH ARTICLE

Pharmaceutical Development and Standardization of Mamajjaka Ghanavati

Shivangni Raj[1], Gopal L. Khatik[2], Anil Kumar Sah[1], Manish Vyas[1], Pramod Yadav[3], P. K. Prajapati[3]

[1]Department of Ayurveda, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab – 144 411, India, [2]Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab – 144 411, India, [3]Department of Rasashastra & Bhaishajya Kalpana, All India Institute of Ayurveda, Sarita Vihar, New Delhi – 110076, India

Abstract

Introduction: Ethnomedicine is contributed to the evolution of different systems of medicine, namely, *Ayurveda*, *Siddha*, *Unani*, and *Naturopathy* including the modern medicine. Enicostemma littorale blume *[E. littorale* (*Mamajjaka)]* is one of the traditional medicines used mainly in Gujarat, Madhya Pradesh, and Rajasthan as a stomachic, tonic, carminative, and appetizer. *It* is generally prescribed in the form of pills (*Ghanavati*) for the treatment of Type 2 diabetes in *Ayurveda*. Recent studies based on the antidiabetic effect of *E. littorale* suggest its role to reduce blood glucose by increasing serum insulin level. However, standards of *Mamajjaka Ghanavati* are not available in the official monographs. **Objective:** The study was designed to develop standards for the preparation and evaluation of *Mamajjaka Ghanavati*. **Results and Discussion:** The formulation is complying with the all standards available for the pills in the Ayurvedic Pharmacopoeia of India including hardness, friability, disintegration, and weight variation. High-performance thin-layer chromatography study revealed the presence of many phytoconstituents in the formulation.

Key words: *Ghanavati*, *Mamajjaka*, standardization

INTRODUCTION

Ethnomedicine is the knowledge based on the curative and palliative effects of certain herbs, animals, and minerals. This knowledge is the outcome of trial and error practices of the several generations. Ethnomedicine is contributed to the evolution of different systems of medicine, namely, *Ayurveda*, *Siddha*, *Unani*, and *naturopathy* including modern medicine. It has continuously providing the information about the various effective drugs for the exploration of their therapeutic profile**.** Enicostemma littorale *blume* (*E. littorale*) is one of the traditional medicines used mainly in Gujarat, Madhya Pradesh, and Rajasthan as a stomachic, tonic, and carminative belonging to the family Gentianaceae[1] and appetizer. *E. littorale* is also prescribed as a single or combination in the form of pills (*Vati*) for the treatment of Type 2 diabetes in *Ayurveda*. Recent studies based on the antidiabetic effect of *E. littorale* suggesting its role to reduce blood glucose and increase serum insulin level. Significant improvement in kidney function, lipid profile, and systolic and diastolic blood pressure also reported.[2] Moreover, it possesses multidimensional therapeutic properties, namely, antimicrobial activity,[3] antihelminthic activity,[4] antinociceptive effect,[5] antioxidant activity,[6] antiulcer activity,[7] anti-inflammatory activity, antitumor activity,[8] hepatoprotective activity,[9] hepatomodulatory activity, and antihyperlipidemic activity including the hypoglycemic activity,[10] antihyperinsulinemic activity,[11] and diabetic neuropathy activity.[12]

The World Health Organization and Ministry of AYUSH (Ministry of Ayurveda, Yoga, Naturopathy, Unani, Siddha, Homeopathy), India, are collaboratively establishing protocols and monographs for the global acceptance of traditional medicines. Standardization confirms the identity,

Address for correspondence:
Dr. Manish Vyas, Department of Ayurveda,
School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab - 144 411, India.
Phone: +91-9780210783. E-mail: vymanish@gmail.com

Received: 11-09-2017
Revised: 20-12-2017
Accepted: 25-12-2017

quality, purity, and efficacy of drugs and formulations.[13] Therefore, continuous efforts of the Ayurvedic Pharmacopoeia Committee and Pharmacopoeial Laboratory for Indian Medicines have resulted in the publication of several monographs for the standardization of Ayurvedic drugs and formulations. However, standards are not available for the *Mamajjaka Ghanavati* which commonly used by the traditional healers and Ayurvedic physicians for the treatment of diabetes. Hence, the present study has been designed to prepare and standardize the *Mamajjaka Ghanavati* using different analytical parameters.

MATERIALS AND METHODS

Collection of plant material

The whole plant of *E. littorale* was collected from the Dang forest, Gujarat, India. The drug was authenticated from the Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar.

Preparation of *Mamajjaka Ghanavati*

The collected plant material was washed with water and shade dried. The shade-dried material was coarsely powdered and sieved through the mesh no. 8. Three major steps were involved in the preparation of *Mamajjaka Ghanavati* including the preparation of decoction *(Kwatha),* concentrate *(Ghana),* and pills *(Ghanavati)*, respectively. A total of six batches of *Mamajjaka Ghanavati* were prepared and observed for the standardization of process. The ratios of drug and water are mentioned in Table 1.

Preparation of decoction (Kwatha)

300 g coarse powder of *E. littorale* was soaked in 16 part of water (4800 ml) for 15 h. Then, it was subjected to the mild heat (80–90°C) and reduced up to one-eighth of its initial quantity.[14] After one-eighth reduction of water, the decoction (*Kwatha*) was filtered through double-folded cotton cloth and collected in a separate vessel.[15] The results and observations of the process are mentioned in Table 2.

Table 1: Ingredients and quantity for the preparation of decoction (Kwatha)

Sr. No.	Ingredients	Quantity
1	E. littorale	300 g
2	Water	4800 ml

Preparation of Ghana

The prepared decoction (*Kwatha*) was further subjected to mild heat (80–90°C) to convert it into semisolid form. In the final stage, indirect heating was given to avoid denaturation of the concentrate *(Ghana)* by heat.[16] The results and observations of the process are mentioned in Table 3.

Preparation of pills (*Ghanavati*)

Pills (*Vati*) from *Ghana* were prepared manually as described in the classical texts.[17] The concentrate *(Ghana)* was mixed with fine power of *E. littorale* and rolled between hands to make the pills (*Vati*) of 500 mg. Then, pills (*Vati*) were placed in oven at temperature 45°C for 12 h for drying. Then, prepared pills *(Ghanavati)* were subjected to various analytical parameters. The results and observations of the process are mentioned in Table 4.

Analysis of *Mamajjaka Ghanavati*

All the batches of the formulation were subjected for organoleptic, physicochemical, qualitative, and high-performance thin-layer chromatography (HPTLC) study to develop standards for the *Mamajjaka Ghanavati*.

Organoleptic evaluation

The individual batch of formulation was evaluated for its organoleptic parameters including color, odor, taste, and texture. The observations are mentioned in Table 4.

Physiochemical evaluation of formulation

The prepared batches of formulation were evaluated on the basis of physiochemical parameters, namely, loss on drying,[18] total ash,[19] acid-insoluble ash, water-soluble ash,[20] alcohol-soluble extractive value,[21] water-soluble extractive value,[22] weight variation,[23] disintegration time,[24] friability,[25] and hardness.[26] The results and observations of the parameters are mentioned in Table 5.

Table 2: Observations and results during preparation of decoction *(Kwatha)*

Parameter	Batch					
	I	II	III	IV	V	VI
Quantity of coarse powder (g)	300	300	300	300	300	300
Volume of water (ml)	4800	4800	4800	4800	4800	4800
Duration of soaking (h)	15	15	15	15	15	15
Duration for the preparation of decoction (min)	135	135	135	135	135	135
Final volume of decoction (ml)	610	630	620	630	615	620

Table 3: Observation and results of concentrate (*Ghana*)

Parameters	Batch						Mean+percentage deviation
	I	II	III	IV	V	VI	
Duration for preparation of concentrate (min)	200	230	210	200	220	210	211±5.54%
Final quantity of concentrate (g)	72.2	70.4	71.6	70.7	71.8	70.9	71.27±0.99%
Percentage yield of concentrate (%)	24.06	23.46	23.86	23.56	23.93	23.63	23.75±0.99%

Table 4: Organoleptic study of formulation

Parameters	Batch					
	I	II	III	IV	V	VI
Color	Blackish-brown	Blackish-brown	Blackish-brown	Blackish-brown	Blackish-brown	Blackish-brown
Odor	Characteristics	Characteristics	Characteristics	Characteristics	Characteristics	Characteristics
Taste	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter
Shape	Round	Round	Round	Round	Round	Round

Table 5: Physicochemical parameters of formulation

Parameters	Batch						Mean±Percentage deviation
	I	II	III	IV	V	VI	
LOD at 105°C (%)	7.09	7.00	7.41	7.27	11.08	11.02	8.48±23.47%
Total ash (%)	11.02	10.89	11.18	11.07	10.94	11.11	11.04±0.97%
Acid-insoluble ash (%)	0.22	0.27	0.30	0.32	0.28	0.18	0.261±19.92%
Water-soluble extract (%)	86.21	84.00	85.06	84.23	86.39	85.09	85.16±1.15%
Alcohol-soluble extract (%)	12.96	13.02	12.87	12.89	13.05	12.95	12.95±0.54
pH	5.6	5.7	5.6	5.5	5.7	5.5	5.6±1.58%
Disintegration test (min)	15:07	15:00	15:05	15:07	15:04	15:01	15.04±0.01%
Friability (%)	0.098	0.097	0.098	0.098	0.097	0.099	0.09±0.72%
Hardness (kg/cm^2)	5.34	5.35	5.35	5.35	5.34	5.35	5.35±0.99%
Weight variation	Pass	Pass	Pass	Pass	Pass	Pass	

HPTLC study of Mamajjaka Ghanavati

50 ml of methanol was added in the 5 g of sample and vigorously shaken for 15–20 min. Then, it was subjected to mild heat for ½ h and filtered after shelf cooling. The filtrate was concentrated on water bath and spotted on a precoated plate with Silica gel GF254 (E. Merck). The test solution was loaded and the solvent system of ethyl acetate:methanol (8:2) was used for development.[27] Developed plate was studied at 254nm by using CAMAG TLC SCANNER 3 system.

In vitro studies

In vitro studies have been carried out to evaluate the antioxidant and antidiabetic property of *Mamajjaka Ghanvati*. OH scavenging assay and DPPH assay were carried out for the antioxidant activity, and α-amylase inhibition assay was carried out for the antidiabetic activity of the formulation.

RESULTS AND DISCUSSION

Mamajjaka Ghanavati is an effective antidiabetic ethnomedicine which is frequently used by traditional healers and Ayurvedic physicians. However, standards of its preparation and evaluation are not available. Therefore, in the present study, standards for the preparation and evaluation of *Mamajjaka Ghanavati* are developed. Six batches of the formulation were prepared and evaluated for the development of standards.

Each batch of the pills (*Ghanavati*) was prepared by the classical method. 300 g of coarse powder of *E. littorale was soaked in* 4800 ml of water *for 15 h to prepare the decoction* [Table 1]. Then, it was subjected to the heat on the mild temperature till the one-eighth quantity of water and filtered. It took around 135 min for the first batch then the same duration was used to prepare decoction (*Kwatha*) for remaining batches. The average yield of the decoction

Table 6: Qualitative tests for formulation

Components	Chemical test	Color/observation	Results
Phenolic compound	-	Brown	–
Tannins	Ferric chloride test	Greenish-black	+
Flavonoids	Shinoda test	Yellow	+
Coumarins	KOH	Yellow	+
Steroidal glycosides	L. Burchard test	Reddish-brown ring	+
Alkaloids	Mayer's test	Red	+
	Dragendorff's test	Red	
Protein	Xanthoprotein test	Yellow	+
Quinones	Sodium hydroxide test	Green	+
Anthraquinone glycoside	Borntrager test	Greenish-brown	–
Saponins	Foam test	Formation of foam	+
Reducing sugars	Fehling's test	Brick red	+
Fixed oil and Fats	Spot test	Oil stain on paper	+

Table 7: Rf values of different compound in the formulation

Rf values
0.03
0.08
0.14
0.24
0.30
0.47
0.57
0.81
0.91

(*Kwatha*) was 620 ± 1.29% ml [Table 2]. Then, prepared decoction was subjected to further heating till the semisolid consistency. The water bath was used at the final stage of concentrate (*Ghana*) to avoid the denaturation of the water-soluble constituent due to the heat. The average yield of concentrate (*Ghana*) was 71.27 ± 0.99% g. The percentage of the yield of concentrate (*Ghana*) was 23.75 ± 0.99%. Approximately 20% of fine powder of *E. littorale* was mixed homogeneously in concentrate (*Ghana*) to overcome from its sticky nature. Then, it was converted into the pills by rolling between hands. The dose of pills was 500 mg. Prepared pills were subjected for the drying in oven at 45°C temperature for 12 h.

Pharmaceutical analysis of each batch of formulation was performed using organoleptic, physicochemical, and qualitative tests. Chromatographic profile of formulation was developed by HPTLC. The organoleptic characteristics were found the same for all the batches [Table 4]. Physicochemical parameters were evaluated for all the batches using the methods described in the Ayurvedic Pharmacopoeia of India. Average results of pH, loss on drying, total ash, acid-insoluble ash, water-soluble extractive value, and alcohol-soluble extractive value were 5.6 ± 1.58%, 8.48 ± 23.47%, 11.04 ± 0.97%, 0.261 ± 19.92%, 85.16 ± 1.15%, and 12.95 ± 0.54, respectively. Pills of all batches were passed in weight variation test (not more than 5%). Average disintegration time for all the batches was 15.04 ± 0.01% min. The average percentage of friability for all the batches was 0.09 ± 0.72%. Moreover, the average hardness for pills was 5.35 ± 0.99% kg/cm^2 [Table 5].

Qualitative analysis of formulation revealed the presence of tannins, flavonoids, coumarins, steroidal glycosides, alkaloids, protein, quinones, saponins, reducing sugars, fat, and fixed oil [Table 6].

Chromatographic profile of formulation revealed the presence of nine compounds with Rf of 0.03, 0.08, 0.14, 0.24, 0.30, 0.47, 0.57, 0.81, and 0.91 [Table 7, Figure 1]. Thus, the formulation is rich in phytoconstituents which may play a role for its wide therapeutic properties including the antidiabetic.

CONCLUSION

Mamajjaka Ghanavati should be prepared using 16 times of water and reduced up to one-eighth to prepared decoction (*Kwatha*) at 90°C temperature. The average yield of concentrate (*Ghana*) was 71.27 ± 0.99% g. Average results of pH, loss on drying, total ash, acid-insoluble ash, water-soluble extractive value, and alcohol-soluble extractive value were 5.6 ± 1.58%, 8.48 ± 23.47%, 11.04 ± 0.97%, 0.261 ± 19.92%, 85.16 ± 1.15%, and 12.95 ± 0.54, respectively. The formulation is complying with the all standards available for the pills in the Ayurvedic Pharmacopoeia of India. The

Figure 1: High-performance thin-layer chromatography plate

qualitative tests and HPTLC profile of *Mamajjaka Ghanavati* revealed the presence of a number of phytoconstituents which may responsible for its wide array of therapeutic properties.

REFERENCES

1. Sankaranarayanan S, Bama P, Ramachandran J, Kalaichelvan PT, Deccaraman M, Vijayalakshimi M, *et al*. Ethnobotanical study of medicinal plants used by traditional users in Villupuram district of Tamil Nadu, India. J Med Plants Res 2010;4:1089-101.
2. Upadhyay UM, Goyal RK. Efficacy of *E. littorale* in Type 2 diabetic patients. Phyto Res 2004;18:233-5.
3. Praveena P, Sudarsanam D. *In vitro* antimicrobial activity studies on *Enicostemma littorale* (Lam), raynal whole plants. Int J Curr Res 2011;11:123-4.
4. Mishra S, Shukla P. *In vitro* anthelmintic activity of *Enicostemma littorale* blume. Int J Pharma Sci Res 2011;2:1193-6.
5. Jaishree V, Badami S, Kumar MP, Tamizhmani T. Antinociceptive activity of swertaimarin isolated from *Enicostema axillare*. Phytomedicine 2009;16:227-32.
6. Mukundray NB, Chauhan K, Gupta S, Pillai P, Pandya C, Jyoti V, *et al.* Protective effect of *Enicostemma littorale* blume methanolic extract on gentamicin induced nephrotoxicity in rats. Am J Inf Dis 2011;7:83-90.
7. Roy SP, Niranjan CM, Jyothi TM, Shankrayya MM, Vishawanath KM, Prabhu K, *et al.* Antiulcer and anti-inflammatory activity of aerial parts of *Enicostemma littorale* Blume. J Young Pharm 2010;2:369-73.
8. Kavimani S, Manisenthilkumar KT. Effect of methanolic extract of *Enicostemma littorale* on dalton's aseptic lymphoma. J Ethnopharmacol 2000;71:349-52.
9. Vaijanathappa J, Badami S, Bhojraj S. *In vitro* antioxidant activity of *Enicostemma axillare*. J Heal Sci 2008;54:524-8.
10. Prince PS, Srinivasan M. *Enicostemma littorale* blume aqueous extract improves the antioxidant status in alloxan-induced diabetic rat tissues. Acta Pol Pharm 2005;62:363-7.
11. Gohil TA, Patel JK, Vaghasiya JD, Manek. Antihyperglycemic and anti hyperinsulinemic effect of aqueous extract of *Aegle marmelos* leaf and *Enicostemma littorale*. Ind J Pharm 2008;40:66-91.
12. Bhatt NM, Barua S, Gupta S. Protective effect of *Enicostemma littorale* blume on rat model of diabetic neuropathy. Am J Infect Dis 2009;5:106-12.
13. Available from: http://www.pib.nic.in/newsite/PrintRelease.aspx?relid=136482. [Last accessed on 2017 Apr 04].
14. Vyas M, Shukla VJ, Patgiri BJ, Prajapati PK. An unique concentrated and fermented dosage form of pravahi kwatha. Int J Pharm Biol Arc 2010;1:287.
15. Shastri A. Sushruta, Sushruta Samhita, Uttaratantra, Edited with Ayurveda Tatva Sandipika Hindi Commentary 24/20. 13th ed. Varanasi: Chaukhamba Sanskrit Sansthan; 2002.
16. Brahmanand T. Sarangadhara Samhita of Pandit Sarngadhar Acarya, Mdhyam Khanda. Varanasi: Chaukhamba Surbharti Prakashan; 2013. p. 210.
17. Sthana PS. Agniveshacharya, Charak Samhita, Elaborated by Charak and Drudhabala Edited by 26/206. Varanasi: Chaukhambha Orientalia; 2007. p. 446.
18. Sanmugarajah V, Thabrew I, Sivapalan SR. Phyto, physicochemical standardization of medicinal plant *Enicostemma littorale*, blume. IOSR J Pharm 2013;3:53.
19. Chaudhari RK, Girase NO. Determination of soluble extractives and physicochemical studies of bark of *Sesbania sesban* (L) Merr. J Chem 2015;7:658.
20. The Ayurvedic Pharmacopeia of India. Government of India. Part I. 1st ed. Vol. II. Apx-2. New Delhi: Controller of Publications; 1999. p. 191.
21. The Ayurvedic Pharmacopeia of India. Government of India. Part I. 1st ed. Vol. II. Apx-2. New Delhi: Controller of Publications; 1999. p. 190.
22. World Health Organization. Quality Control Methods for Medicinal Plants Materials. Geneva: World Health Organization; 1998. p. 1-115.
23. Available from: https://www.jstor.org/stable/2347313?seq=1#page_scan_tab_contents. [Last accessed on 2017 May 08].
24. Available from: http://www.plimism.nic.in/Protocol_For_Testing.pdf. [Last accessed on 2017 May 08].
25. Mathur N, Kumar R. Evalution of quality Control Parameters on various brands of paracetamol tablet formulation. World J Pharm Pharm Sci 2015;4:982.
26. Lachman L, Lieberman HA, Kanig JL. The Theory and Practice of Industrial Pharmacy. Special Indian Edition. Bombay: Varghese Publishing House; 2009. p. 299.
27. Praveena P, Sudarsanam D. Thin layer chromatographic method for quantification of swertiamarin in whole plant powder of *Enicostema littorale.* Int J Pharmacog Phytochem Res 2012;4:64-8.

Source of Support: Nil. **Conflict of Interest:** None declared.