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Pharmaceutical Standardization

Pharmaceutical standardization of *Mamajjaka* (*Enicostemma littorale* Auct. non Bl) *Ghana*

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Abstract

Mamajjaka (*Enicostemma littorale* Auct. non Bl) is a well known folklore medicine frequently used for the treatment of *Madhumeha* (diabetes mellitus). There is no direct reference available for its antihyperglycaemic activity in Ayurvedic classics. Considering this, a study is planned towards developing pharmaceutical standardization of *Mamajjaka Ghana*. In this study, five batches of *Mamajjaka Ghana* were prepared and findings were systematically recorded to maintain the Standard Operating Procedure (SOP). An average of 14.78% *Ghana* was obtained. The physico-chemical parameters, qualitative test for various functional groups, quantitative estimation of total alkaloids, HPTLC profile, heavy metal analysis and microbial overload were carried out of *Mamajjaka Ghana*.

Key words: *Enicostemma littorale, Ghana, Kwatha, Mamajjaka*

Introduction

Considering the significance of traditional practices in global health care, WHO has been encouraging and promoting traditional practices since past few decades. Hence, the standardization of raw drugs, processing, finished products, verification of the claims, mechanism of action, products that are free from heavy metal and microbial contamination, etc., have became major issues, which are to be taken into consideration in order to increase the global acceptability of herbal drugs and also to prove their respective clinical efficacy. It is very tedious to standardize *Ayurvedic* herbal formulations due to number of factors. The non availability of reference standards also hurdles the study. In spite of that, the present task is undertaken to evaluate and to compare the formulation with the available physicochemical parameters.

Mamajjaka (*Enicostemma littorale* Auct. non Bl) is one of the herbs used in *Madhumeha* traditionally in Gujarat, Madhya Pradesh, and Rajasthan. *Ghana* is a widely acceptable dosage form in the present scenario due to its advantages like palatability, shelf life, easy administration, etc. Keeping this in view, *Mamajjaka Ghana* was prepared to develop Standard Operating Procedure (SOP) and analytical profile.

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Materials and Methods

Collection of raw materials

The raw drug was procured from Pharmacy, Gujarat Ayurved University, Jamnagar, Gujarat, India, and authenticated at the Pharmacognosy Laboratory, IPGT and RA, Gujarat Ayurved University, Jamnagar. *Mamajjaka* was powdered in grinding mill and passed through sieve no. 8. Following general principles, 2kg coarse powder of *Mamajjaka* was added with 16 parts (32 l) of potable water[1] in a stainless steel vessel and the contents were soaked overnight (12 h). The next day, *Kwatha* was prepared by applying constant mild heat to the mixture to facilitate evaporation and intermediate stirring was carried out till the volume reduced up to one-eighth of the initial quantity. After desirable reduction of volume, the *Kwatha* was filtered through four folded cotton cloth and collected in a separate vessel for further processing. The residue was discarded.

The prepared *Kwatha* was subjected to further heating on a gas stove maintaining the temperature between 70°C and 75°C till a semisolid consistency was obtained. Then the material was shifted into a glass tray and placed in a hot-air oven at 45°-50°C for complete drying. After complete drying, the content were grinded in a mixer grinder, collected in duly labeled air-packed glass bottle. Further four additional batches were prepared in order to ensure the Standard Manufacturing Procedure.

Table 1: Observation and result obtained during preparation of *Mamajjaka Kwatha*

Parameters	Batches					Mean
	I	II	III	IV	V	
Initial qty. of *Kwatha Churna* (g)	2000	2000	2000	2000	2000	2000
Total quantity of water (l)	32.00	32.00	32.00	32.00	32.00	32.00
Total time of soaking (h)	12	12	12	12	12	12
Temp. during the preparation of *Kwatha* after 1 h (°C)	80-90	80-90	80-90	80-90	80-90	80-90
Total time taken for 1/8th reduction (h)	09.00	09.05	09.10	09.00	09.15	09.06
Total quantity of *Kwatha* obtained (l)	4.14	4.15	4.15	4.16	4.14	4.15
Wt. of residue after filtration (g)	2260	2700	2720	2765	2568	2602.06

Table 2: Observations and results obtained during preparation of *Mamajjaka Ghana*

Parameters	Batches					Mean
	I	II	III	IV	V	
Mamajjaka Kwatha (ml)	4100	4100	4100	4100	4100	4100
Total time taken for the preparation of *Ghana* (h)	7.45	7.50	8.10	7.55	8.00	7.56
Final quantity of *Ghana* obtained before drying (g)	700.2	686.4	712.6	696.2	704.3	699.94
Total time for drying (h)	26	25	27	25	26	25.8
Final quantity of dried *Ghana* obtained (g)	296.6	288.4	302.2	290.2	300.6	295.6
Percentage of dried *Ghana* obtained (%)	14.83	14.42	15.11	14.51	15.03	14.78

Table 3: Organoleptic characteristics of *Mamajjaka Kwatha Churna*

Parameter	Result
Rupa	Greenish brown
Rasa	Bitter
Gandha	Characteristic
Sparsha	Rough

Table 4: Physico-chemical data of *Mamajjaka Kwatha Churna*

Parameter	Values (%)
Loss on drying	0.198 w/w
Ash value	11.96 w/w
Water soluble extractive	27.65 w/w
Alcohol soluble extractive	24.57 w/w

Table 5: Organoleptic characteristics of *Mamajjaka Kwatha*

Parameter	Batches				
	I	II	III	IV	V
Colour	Brownish	Brownish	Brownish	Brownish	Brownish
Smell	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Touch	Liquid	Liquid	Liquid	Liquid	Liquid
Taste	Bitter	Bitter	Bitter	Bitter	Bitter

Table 6: Physico-chemical data of *Mamajjaka Kwatha*

Parameter	Batches					Mean
	I	II	III	IV	V	
pH value	4.80	4.53	4.57	4.72	4.61	4.65
Specific gravity	1.0061	1.0053	1.0024	1.0038	1.0098	1.0055
Total solid contents (% w/w)	5.71	5.47	5.45	5.55	5.67	5.57

Analytical study

Mamajjaka Kwatha Churna, *Mamajjaka Kwatha*, and *Mamajjaka Ghana* were analyzed by employing various analytical parameters. Organoleptic characteristics (color, odor, taste) and physico-chemical analysis like loss on drying at 110°C,[2] ash value,[3] acid insoluble ash,[4] pH value,[5] specific gravity at 40°C,[6] total solid content,[7] water soluble extractives,[8] methanol soluble extractives[9] were carried out. *Mamajjaka Ghana* was subjected to further higher analysis, namely, qualitative test for various functional groups[10,11] and quantitative estimation of total alkaloids.[12] Tests for presence of Heavy metals[13] (lead, arsenic, and mercury) and Microbial contamination[14] (a bacterial and fungal growth study) were carried out.

HPTLC profile

Test solution[15]: Five grams of the finely powdered plant material was defatted with 25 ml of solvent ether and then refluxed with 25 ml of methanol for 25 min on water bath consecutively three times; it was filtered and the solvent was removed under reduced pressure. Then 25 mg of the extractive was dissolved

Table 7: Organoleptic characteristics of *Mamajjaka Ghana*

Parameter	Batches				
	I	II	III	IV	V
Colour	Brown	Brown	Brown	Brown	Brown
Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Taste	Bitter	Bitter	Bitter	Bitter	Bitter
Touch	Smooth	Smooth	Smooth	Smooth	Smooth

Table 8: Physicochemical data of *Mamajjaka Ghana*

Parameter (%)	Batches					Mean
	I	II	III	IV	V	
pH value	4.90	4.60	4.80	4.70	4.65	04.73
Loss on drying	8.87	8.03	9.10	8.96	8.66	08.72
Ash value (w/w)	11.65	10.08	12.83	11.75	10.43	11.35
Acid insoluble ash (w/w)	0.29	0.27	0.29	0.28	0.27	00.28
Water soluble extract (w/w)	16.19	13.75	15.89	14.59	16.80	15.44
Alcohol soluble extract (w/w)	12.34	17.22	15.75	14.34	15.28	14.99

Table 9: Qualitative tests for various functional groups of *Mamajjaka Ghana*

Functional groups tested	Batches				
	I	II	III	IV	V
Alkaloids	+Ve	+Ve	+Ve	+Ve	+Ve
Glycosides	+Ve	+Ve	+Ve	+Ve	+Ve
Starch	–Ve	–Ve	–Ve	–Ve	–Ve
Tannin	–Ve	–Ve	–Ve	–Ve	–Ve
Saponin	+Ve	+Ve	+Ve	+Ve	+Ve
Flavanoids	+Ve	+Ve	+Ve	+Ve	+Ve
Phenols	–Ve	–Ve	–Ve	–Ve	–Ve

+Ve: Present, -Ve: Absent

Table 10: Percentage of the total alkaloid content of *Mamajjaka Ghana*

Sample	Total alkaloid content (%)
Mamajjaka Ghana	0.009

Table 11: Pathogen and total microbial count of *Mamajjaka Ghana*

Samples	Pathogen	
	Bacterial	Fungus
Mamajjaka Ghana	Absent	Absent

Table 12: Heavy metal analysis of *Mamajjaka Ghana,*

Sample	Element		
	Mercury as Hg	Arsenic as As	Lead as Pb
Mamajjaka Ghana	Not detected	Not detected	Not detected

in 10 ml of methanol. Toluene: ethyl acetate: formic acid: methanol (6:3:0.1:1% v/v) was selected as the solvent system after multiple trial-and-error method. The developed plate was visualized under visible daylight, short UV (254 nm), long UV (366 nm), and after spraying with the anisaldehyde–sulfuric acid reagent was again observed in daylight.

Results and Discussion

Initially, liquid of the *Mamajjaka Kwatha* was light brown in color and bitter in taste. Evaporation was started at 70°C, which was aggravated on stirring. An average 9.06 h time was required for the preparation of *Mamajjaka Kwatha*. After 2 h heating of *Mamajjaka Kwatha*, mild sticky nature was observed. After drying in the oven, the dark brown semisolid material converted into a blackish brown solid material.

The degree of size reduction depends upon the structure of the drug. Particle size reduction facilitates adequate mass transfer for better extraction. To facilitate further extraction of water soluble constituents, overnight soaking was done, which allow micelles to take up liquid film and tissue swelling. Swelling also results from distension and bursting of thin-walled cells that have taken up the liquid by osmosis. During processing, the application of mild heat is required with occasional stirring to avoid destruction of the components sensitive to higher temperature. During the preparation of *Mamajjaka Kwatha*, maximum temperature was between 90°C and 95°C.

Kwatha was further reheated and converted into a semisolid material, to remove watery portion; in the conversion state, (from semisolid to solid) continuous, mild heat (70-75°C) was applied and stirring was done very cautiously to avoid burning of the material. After 3 h of heating, the liquid became stickier. After attaining semisolid consistency, the contents were transferred into a glass tray and were spreaded as a uniform layer. Finally, for complete drying, the tray was placed in an oven at 45-50°C. After complete drying, the contents were collected and stored in air tight container for further use. The average time required for the preparation of *Kwatha* was 9.06 h, while for *Ghana* it was 7.56 h. The average yield of *Kwatha* and *Ghana* was 4.15 l and 295.6 g (14.78%), respectively. The observations of *Kwatha* and *Ghana* preparation are placed at Tables 1 and 2.

The organoleptic characters of *Mamajjaka Kwatha Churna* are tabulated at Table 3. Observations of the analytical parameters are tabulated at Tables 4-8.

Qualitative tests were done to detect the presence of functional groups. The study reveals the presence of alkaloids, glycosides, saponins and flavonoids in all five batches of both the

Table 13: HPTLC profile of *Mamajjaka Ghana*

Sample	254 nm		366 nm		After spraying	
	No. of spots	R_f	No. of spots	R_f	No. of spots	R_f
Mamajjaka Ghana	13	0.03, 0.12, 0.18, 0.22, 0.27, 0.31, 0.36, 0.46, 0.49, 0.55, 0.73, 0.87, 0.94	8	0.04, 0.12, 0.20, 0.38, 0.46, 0.49, 0.73, 0.95	7	0.2, 0.5, 2.0, 2.8, 4.3, 5.9, 3.6

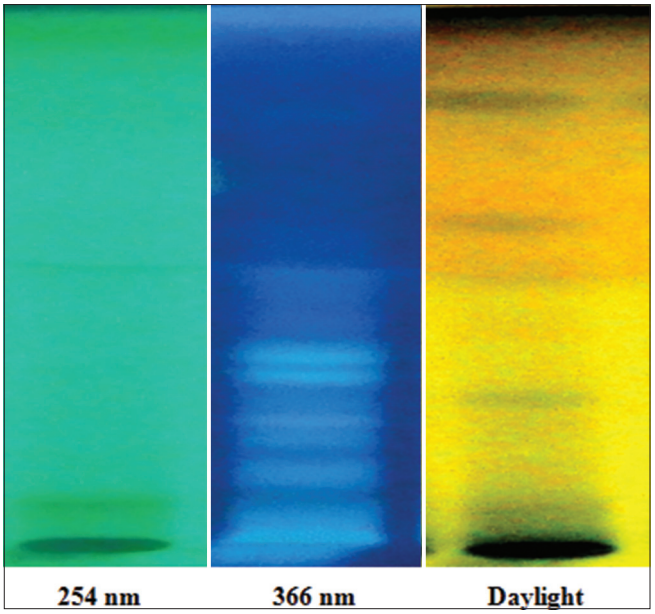

Figure 1: Visualization of *Mamajjaka Ghana* at 254 nm, 366 nm, and in daylight

formulations whereas an absence of starch, tannin, and phenol was observed [Table 9]. The total alkaloid content observed in *Mamajjaka Ghana* was 0.009% [Table 10]. Observations on microbial contamination and heavy metal analysis were found to be in permissible limits [Tables 11 and 12]

In the absence of the marker compound, the HPTLC profile of *Mamajjaka Ghana* was attempted [Figures 1 and 2]. Visual spots and R_f values were recorded [Table 13]. This particular study can be considered as standard for further research work.

Conclusion

Mamajjaka is a widely used traditional herb in the present era for its antidiabetic and antipyretic attributes. The pharmaceutical standardization of this herb ensures the uniformity of the preparation, thereby proving its global acceptance. Physicochemical and phyto-chemical data as well as HPTLC, heavy metal profile, and microbial overload are essential parameters followed to develop SOP. The data generated by this particular work can be taken as standard for the preparation of *Mamajjaka Ghana* at a laboratory scale.

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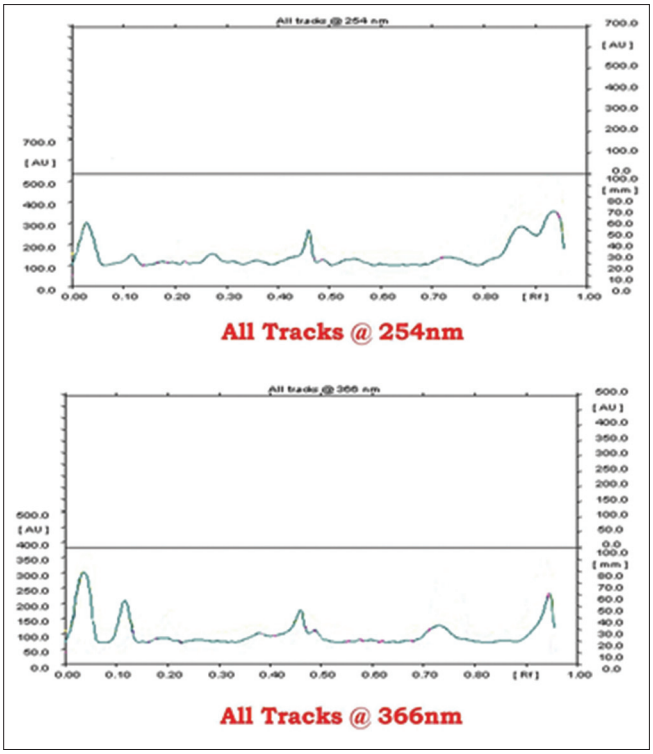

Figure 2: Densitometry of *Mamajjaka Ghana* at 254 nm and 366 nm

References

1. Sharangadhara, Sharangadhara Samhita, Madyama Khanda, editor: Pandit Parasuram Shastri, Vidyasagar, Chaukhamba Orientalia, Varanasi, 6th ed. 2005, 2/1, p. 144.
2. Anonymus. The Ayurvedic Pharmacopoeia of India, Ministry of Health and Family Welfare, Gov. of India, Part 1, Vol. I, 1st ed. 1999. p. 214(2.2.9).
3. Ibid, The Ayurvedic Pharmacopoeia of India, Part -1, Vol-1, Appendix-2, p.-213 (2.2.3).
4. Ibid, The Ayurvedic Pharmacopoeia of India, Part -1, Vol-1, Appendix-2, p.-213 (2.2.4).
5. Ibid, The Ayurvedic Pharmacopoeia of India, Part -1, Vol-1, Appendix-2, p.-230 (3.3).
6. Ibid, The Ayurvedic Pharmacopoeia of India, Part -1, Vol-1, Appendix-2, p.-236 (3.7).
7. Ibid, The Ayurvedic Pharmacopoeia of India, Part -1, Vol-1, Appendix-2, p.-214 (2.2.9).
8. Ibid, The Ayurvedic Pharmacopoeia of India, Part -1, Vol-1, Appendix-2, p.-214 (2.2.7).
9. Ibid, The Ayurvedic Pharmacopoeia of India, Part -1, Vol-1, Appendix-2, p.-214 (2.2.6).
10. Baxi AJ, Shukla VJ, Bhatt UB. Methods of qualitative testing of some Ayurvedic formulations, Gujarat Ayurved University, Jamnagar, 1st ed.2001, p. 5-12.
11. Khandelwal KR, Practical Pharmacognosy Techniques and Experimental, Nirali Prakashan, Pune, 16th ed. 2006, p. 149-56.
12. Sim Stephen K,Medicinal plant alkaloids: An introduction for Pharmacy

students, University of Toronto press, 2nd ed. 1965, p. 44.
13. Anonymous. Quality control methods for medicinal plant materials, W.H.O., Pharm, 1992, 559, Rev. 1, p. 46-48.
14. Anonymous. Quality control methods for medicinal plant materials, W.H.O., (An Authorized publication of World Health Organization, Geneva) New Delhi: A.I.T.B.S. Publishers and Distributors (Regd.); 2002, p. 48-55.
15. Quality standards of Indian Medicinal Plants, Vol-3, Indian Council of Medical Research, New Delhi, 1st ed. 2005, P. 208.

हिन्दी सारांश

मामज्जक घन की निर्माण प्रक्रिया का मानिकीकरण

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मामज्जक परंपरागत रूप से मधुमेह में प्रयुक्त औषधि है । यद्यपि इसका कोई ग्रन्थाधार प्राप्त नहीं होता फिर भी वह पारम्परिक रूप से घरेलू उपचार में लम्बे समय से उपयोग में आता रहा है । प्रस्तुत शोधपत्र में मामज्जक द्वारा बनाए गये घन का निर्माणात्मक मानिकीकरण किया गया है । इसके पूर्व शुष्क मामज्जक का वानस्पतिक विश्लेषण द्वारा निर्धारण किया गया । मानिकीकरण के लिये मामज्जक घन का निर्माण पांच वर्गों में किया गया । प्रत्येक वर्ग की निर्माण प्रक्रिया का लिखित ब्यौरा रखा गया । औसतन शुष्क मामज्जक चूर्ण से १४.७८% मामज्जक घन की प्राप्ति हुई ।