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ORIGINAL RESEARCH ARTICLE

Physico-phytochemical Evaluation of Aqueous extract of Mamajjaka [*Enicostemma littorale* auct. non Bl]

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ABSTRACT

Mamajjaka (*Enicostemma littorale auct*. non Bl) is a well-known folklore medicine for the treatment of Madhumeha (Diabetes Mellitus). The drug has been attribute with other therapeutic potentials like antihelmintic, anti-inflammatory, hepatoprotective, antiperiodic etc. In present study analytical profile of aqueous extract (Ghana) of Mamajjaka is carried out with all the available methods including Microbial count, Heavy metal analysis and High performance thin layer chromatographic study. The generated information of the present study will provide data which is helpful in the identification and authentification of the drug Mamajjaka aqueous extract.

Keywords: Mamajjaka Kwatha (Decoction), Mamajjaka Ghana (Aqueous extract)

INTRODUCTION

Considering the significance of traditional practices in global health care, WHO also has been encouraging and promoting these traditional practices since last few decades. Hence, the standardization of raw drug, processing, finished products, verification of the claims, mechanism of action and free from heavy metal and microbial contamination etc. become some of the major issues, which have to be taken into consideration in order to increase the world wide acceptability of herbal drugs throughout the globe and also prove the clinical efficacy of the old age remedies.

Mamajjaka (Enicostemma littorale auct. non Bl) is a familiar herb used in Madhumeha particularly in the state of Gujarat, Madhya Pradesh and Rajasthan. It is used as a substitute for Chirayata (Swertia chirayita Roxb. Lyons.). It is bitter, acrid, thermogenic, liver tonic. It is useful in dyspepsia, helminthiasis, liver disorders, glycosuria and intermittent fevers etc. Aqueous extract is widely acceptable dosage forms in present scenario due to its advantages like palatability, shelf life and easy administration etc. That's why Mamajjaka aqueous extract is prepared for present study.

It is very tedious to standardize Ayurvedic herbal formulations because of a number of factors. Non-availability of reference standards also hurdles the study. In spite of that, the present task is undertaken to evaluate the formulation with the available physico-chemical parameters. Here, an attempt has been made to developed analytical profile of Mamajjaka aqueous extract.

MATERIALS AND METHODS

Preparation of Mamajjaka aqueous extract

Raw drug (Mamajjaka) was collected from Gujarat Ayurveda University Pharmacy, Jamnagar, and authentified by the Pharmacognosy lab. It was powdered in grinding mill and passed through mesh no. 08.size, than it was soaked in 16 times of water and made decoction of it by reducing 1/8 part. The decoction was reheated till it became semisolid and dried in oven at 55°C

Analysis of raw material, intermediate and finished product

Raw drug Mamajjaka course powder, in process formulation Mamajjaka decoction and finished product Mamajjaka aqueous extract were analysed by employing various related analytical parameters, like

A. Organoleptic characteristics: Colour, odour, taste, form were observed.

B. Physico-chemical analysis: Loss on drying at 110°C, ash value, acid insoluble ash, pH value, specific gravity at 40^0C, total solid content, water

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soluble extractives, methanol soluble extractives were carried out.

Mamajjaka aqueous extract was subjected for further higher analysis viz.

A. Qualitative test for various functional groups

B. Quantitative estimation of Total Alkaloids: Observation and results of the experiment are placed in **Table No 1 - 6.**

C. HPTLC profile:

Test solution: Defat 5 gm of the finely powdered plant material with 25 ml of solvent ether then reflux with 25 ml of methanol for 25 min. on water bath consecutively 3 times, filter and remove the solvent under reduced pressure. Dissolve 25 mg of the extractive in 10 ml of methanol.

Toluene**:** Ethyl acetate**:** Formic acid**:** Methanol (6**:**3**:**0.1**:**1%v/v) was selected as solvent system after multiple trials and error method. The developed plate was visualised under visible day light, short UV (254 nm), long UV (366 nm) and after spraying with Anisaldehyde-sulphuric acid reagent and again observed in daylight. The R_f values are recorded **(Table No. 9).**

D. Microbial overload

Bacterial and fungal growth study was carried out **(Table No. 7).**

E. Heavy metal analysis

Lead, Arsenic and Mercury were analysed **(Table No.8)**

OBSERVATIONS AND RESULTS

- The Organoleptic characters of the Mamajjaka course powder, Mamajjaka decoction and wet and dried Mamajjaka aqueous extract sample are tabulated as **Table no. 1.**
- The Physicochemical parameters of the Mamajjaka course powder are tabulated as **Table no. 2.**
- The comparative analytical parameters of Mamajjaka decoction are tabulated as **Table no. 3.**
- The average physicochemical parameters of the Mamajjaka aqueous extract are tabulated as **Table no. 4.**
- The qualitative test for various functional groups is tabulated as **Table no. 5.**
- The % of Total Alkaloid Content is tabulated as **Table no. 6.**
- The total microbial count results are tabulated as **Table no. 7**.
- The results of Heavy metal analysis (I.C.P.M.S.) are tabulated as **Table no. 8**.
- The HPTLC profile of the Mamajjaka aqueous extract is tabulated as **Table no. 9**.

Table 1.Organoleptic characters of Raw (Mamajjaka course powder), Intermediate product (Decoction) and Finish product (Aqueous extract)

S.No.	Parameter	Raw	Intermediate product Decoction	Finished product aqueous extract
1.	Colour	Greenish brown	Brownish	Light Brown
2.	Odour, Texture	Characteristic	Characteristic	Characteristic
3	Touch	Coarse	Liquid	Smooth
4	Taste	Bitter	Bitter	Bitter

Table 2 Physicochemical parameters of Mamajjaka course powder

S. No.	Parameter	Values (% w/w)
1.	Loss on drying	0.198
2.	Ash value	11.96
3.	Water soluble extractive	27.65
4.	Alcohol soluble extractive	24.57

Table 3 Analytical data of Mamajjaka decoction

S.No.	Parameter	Batches					Avg.
		I	II	III	IV	V	
1.	pH	4.80	4.53	4.57	4.72	4.61	4.65
2.	Sp. Gravity	1.0061	1.0053	1.0024	1.0038	1.0098	1.0055
3.	Total solid content (%w/w)	5.71	5.47	5.45	5.55	5.67	5.57

Table 4 Physicochemical parameters of Mamajjaka aqueous extract

S.No.	Parameter	Batches					Avg.
		I	II	III	IV	V	
1.	pH value	4.90	4.60	4.80	4.70	4.65	04.73
2.	Loss on drying	8.87	8.03	9.10	8.96	8.66	08.72
3.	Ash value (%w/w)	11.65	10.08	12.83	11.75	10.43	11.35
4.	Acid insoluble ash (%w/w)	0.29	0.27	0.29	0.28	0.27	00.28
5.	Water soluble extract (%w/w)	16.19	13.75	15.89	14.59	16.80	15.44
6.	Alcohol soluble extract (%w/w)	12.34	17.22	15.75	14.34	15.28	14.99

Table 5 Qualitative tests for various functional groups of Mamajjaka aqueous extract

S.No.	Tests for	Batches				
		I	II	III	IV	V
1.	Alkaloids	+Ve	+Ve	+Ve	+Ve	+Ve
2.	Glycosides	+Ve	+Ve	+Ve	+Ve	+Ve
3.	Starch	-Ve	-Ve	-Ve	-Ve	-Ve
4.	Tannin	-Ve	-Ve	-Ve	-Ve	-Ve
5.	Saponin	+Ve	+Ve	+Ve	+Ve	+Ve
6.	Flavanoids	+Ve	+Ve	+Ve	+Ve	+Ve
7.	Phenols	-Ve	-Ve	-Ve	-Ve	-Ve

+Ve =present, -Ve =absent

Table 6 Percentage of Total Alkaloid Content of Mamajjaka aqueous extract,

Sample	% Total Alkaloid content
Mamajjaka aqueous extract	0.009

Table 7 Pathogen and total microbial count of Mamajjaka aqueous extract,

Samples	Pathogen	
	Bacterial growth	Fungal growth
Mamajjaka aqueous extract	Absent	Absent

Table 8 Heavy Metal Analysis of Mamajjaka aqueous extract,

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

Table 9 HPTLC Profileof methanolic extract of Mamajjaka course powder (MC) and Mamajjaka aqueous extract (MA)

Sample	Short UV 254nm		Long UV 366 nm		After spraying	
	No. of spots	R_f	No. of spots	R_f	No. of spots	R_f
MC	10	0.03, 0.15, 0.21, 0.39, 0.48, 0.51, 0.57, 0.75, 0.88, 0.93	10	0.04,0.09,0.15, 0.21,0.39, 0.47,0.57,0.66,0.75, 0.94	9	0.2, 0.5, 0.9, 1.2, 1.5, 1.8, 2.1, 5.7, 6.1
MA	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

DISCUSSION

The present analytical study has been carried out to establish the quality control parameters of the drug- Mamajjaka aqueous extract. Organoleptic and physicochemical analysis were carried out at three different stages of manufacturing i.e. raw, intermediate process and finished product level, to establish the standard quality parameters for the formulation. Qualitative tests for various chemical moieties carried out to reveal any possible changes from raw to finished product level. HPTLC study was carried out to establish the fingerprinting profile for the formulation and to reveal the possibly active phyto-constituents compare them both at raw and finished product level. Also the microbiological study and heavy metal analysis of Mamajjaka aqueous extract were carried out finally tofulfil and establish the quality standard at finished product level.

The organoleptic characters of Mamajjaka aqueous extract were as shown in **Table No.1**, which were performed at three stages of production because these parameters were changed at different stages. The colour of Mamajjaka course powder was greenish brown while coarse in touch and bitter in taste. The organoleptic characters of Mamajjaka decoction were brownish in colour and non-sticky in touch as shown in **Table No.1**, which differs from the organoleptic characters of Mamajjaka aqueous extract. The colour of samples varies from brownish to light brown due to the effect of heat on the sample.

pH was taken of decoction and different samples of Mamajjaka aqueous extract. **Table No. 3** shows the average pH of decoction was 4.65 of batch 1-5.Table No.4 shows the pH of different samples of Mamajjaka aqueous extract. Those values show not much difference in the pH.

Data pertaining to **Table No.3** shows the slight variation in specific gravity of Mamajjaka decoction. Average specific gravity of decoction was 1.0055.

Data pertaining to **Table No.3** shows the slight variation in total solid content of Mamajjaka decoction. The average total solid content comes 5.71, 5.47, 5.45, 5.55 and 5.67 in batch-1 to batch-5 respectively. In batch-1total solid content comes slight more in comparison to other batches due to more water-soluble extractive comes in Decoction on heating.

Table No. 2 shows loss on drying value of Mamajjaka decoction Churna is 0.19% w/w.

Table No. 4 shows the variation in loss on drying values of Mamajjaka aqueous extract. The average value of loss on drying of aqueous extract was 8.72% w/w.Loss on drying values was high in all the samples of aqueous extract may be due to hygroscopic nature

Ash value of Mamajjaka course powder is 11.96 % w/w as shown in **Table No. 2.** The average ash valueis11.35% w/w in Mamajjaka aqueous extracts samples. (Table No. 4).

The average acid insoluble ash value is 0.28 % w/win Mamajjaka aqueous extract samples. (**Table No. 4**).

The water-soluble extractive value of Mamajjaka course powder is 27.65 % w/w (Table No. 2).The average water-soluble extractive value is 11.35 %w/w in Mamajjaka aqueous extract samples (**Table No. 4**).

The alcohol soluble extractive value of Mamajjaka course powder is 24.57 % w/w (**Table No. 2**).The average alcohol soluble extractive value is14.99 %w/w Mamajjaka aqueous extract samples (**Table No. 4**).

Table No. 5 shows the results of the qualitative test carried out for various functional groups in the finished products. Presence of major known of raw drugs into the finished product suggests the extraction of these moieties in the formulation.Qualitative tests were used to detect the presence of functional groups, which plays very important role in the expression of biological activity. Present study reveals that the presence of alkaloids, glycosides,saponins,flavonoids in all five batches of both the formulations; whereas absence of starch, tannin and phenol were observed.

The percentage total alkaloids content are 0.009 % in Mamajjaka aqueous extract as shown in (**Table No. 6**).

Total Microbial count (**Table No. 7**) and Heavy metal content (**Table No. 8**) of the Mamajjaka aqueous extract were within permissible limit. So the formulation can be considered as free form Microbial and Heavy metals contamination. It may also be inferred that the material was not affected by any contaminants during the preparation.

In absence of marker compound, HPTLC profile of Mamajjaka aqueous extract was attempted (**Fig.No.1 & 2**).

CONCLUSION

Mamajjaka is a widely used traditional herb in present era for its antidiaebetic and antipyretic attributes. Physicochemical and phytochemical data as well as HPTLC, Heavy metal profile and Microbial overload are essential parameters can ensure quality assurance. The data generated by this particular study can be taken as quality control parameter for Mamajjaka aqueous extract.

Fig.-1 Showing visualisation of Mamajjaka aqueous extract at 254 nm, 366 nm and day light

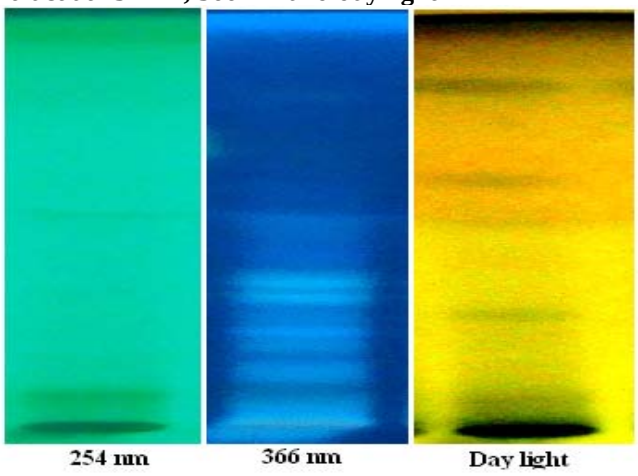
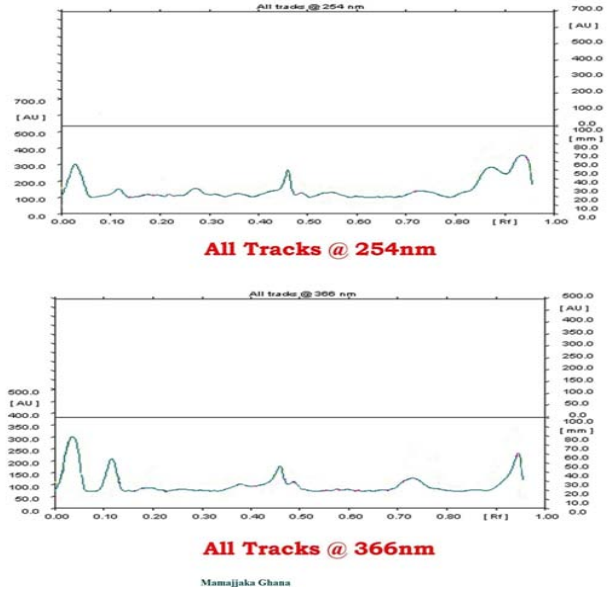


Fig.-2 shows densitometry of Mamajjaka aqueous extract in 254 nm and 366 nm.



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