Symposium

Quality control of an antipsoriatic ayurvedic herbal Formulation: Lajjalu Keram

Abstract

Background: Psoriasis is an autoimmune disorder, which affects a large group of human population of world (3%). Till date, there is no treatment for psoriasis except some herbal drugs and its constituents. Since Ayurveda is the main traditional system of medicine in India, here, we have selected one ayurvedic formulation - Lajjalu Keram, which has been used since long for their quality control. **Methods:** Total microbial load of formulations were carried out for total fungal count and total bacterial count. Lajjalu Keram was also tested by high-performance liquid chromatographic (HPLC) for aflatoxins (B1, B2, G1, and G2), which showed its presence below the permissible limit; similarly, pesticides residues were analyzed using gas chromatography/mass spectrometry for organophosphates and organochlorides, which showed that pesticides were below detection limit (0.1 ppb). The content of heavy metals was analyzed using AAS, which demonstrated the presence of cadmium, lead, and arsenic below permissible limit, whereas mercury was found absent. **Results:** The result of quality control analysis showed the presence of alkaloids, tannins, carbohydrate, saponins, proteins and amino acids, lipid/fats, phenolic compounds, and flavonoids in formulation. The dermal toxicity (LD50) of Lajjalu Keram in Wistar rats was found more than 2000 mg/kg (safe for the management of psoriasis). Formulation was also analyzed for their composition of fatty acids. It was found to have 13 fatty acids, out of which, seven were saturated fatty acids (95.2%) and the rest were unsaturated fatty acids (3.27%). A rapid HPLC method for quantification of mimosine (an unusual amino acid present in formulation) has been developed and validated. The mimosine content in Lajjalu Keram was found to be 0.0070% w/w with % relative standard deviation of 0.41. **Conclusion:** The formulation afforded significant and better protection of carrageenan-induced rat paw edema (72.11% inhibition) as compared to control. The shelf life studies on antipsoriatic herbal formulations were carried out for 6 months at different time intervals. No significant variation in analysis parameters was observed on the storage of formulations up to 6 months. The assay of constituents using mean curve of sigma plot showed 44.2 month of shelf life for Lajjalu Keram.

Key words:
Aflatoxin, gas chromatography-mass spectrometry, high-performance liquid chromatographic, Lajjalu Keram, quality control

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Introduction

Psoriasis is an autoimmune disorder, which affects a large group of human population of world (3%). Till date, there is no treatment for psoriasis except some herbal drugs and its constituents. Since Ayurveda is the main traditional system of medicine in India, here, we have selected one ayurvedic

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formulation - Lajjalu Keram, which has been used since long for the management of psoriasis.

Materials and Methods

Standardization and phytochemical investigation

Lajjalu Keram was standardized as per standard protocol (WHO and AYUSH). Phytochemical screening was performed as per I.P. 1996, by taking whole extract. Results are summarized in Table 1.

Chemical toxicity evaluation

Aflatoxin

Extraction of Aflatoxin was carried out as per AOAC official method.[1] The extracted aflatoxin was de-rivatized and analyzed by high-performance liquid chromatographic (HPLC) (Shimadzu, Japan; Column: C_{18} 15 cm × 4.6 mm; flow rate: 1 mL min^{-1}; Solvent: Water/Acetonitrile/Methanol: 70/17/17; Detector: Fluorescent detector [Table 1].

Pesticides

Extraction of pesticide was also carried out as per AOAC official method.[1] The formulation was dried in rotary evaporator (Buchi, Lab-scale Rotavapor® R-210/R-215) and concentrated up to 5 mL and analyzed by gas chromatography (GC)-mass spectrometry (Perkin Elmer Clarus 500) [Table 1].

Table 1: Results of various standardization parameters for quality control of Lajjalu Keram

Parameter	Results
Color	Greenish
Odor	Smell of coconut oil
Taste	Bitter
Rancidity	No rancidity
Density (weight/ml)	0.917 ± 0.005
Refractive index	1.454 ± 0.001
Viscosity (cps)	55.267 ± 0.973
Acid value	1.267 ± 0.050
Saponification value	30.170 ± 2.236
Ester value	28.903 ± 2.287
Total bacterial count	Nil
Total fungal count	2800 count/g
Heavy metals analysis (limit Cd = 0.3 ppm; As = 3 ppm; Hg = 1 ppm; Pb = 1 ppm)	Cd, As, Hg and Pb, below permissible limit
Aflatoxins (limit - B1 and G1 = 0.5 ppm; B2 and G2 = 0.1 ppm)	B1, B2, G1 and G2, below permissible limit
Pesticides (limit - 0.02-4.0 ppm)	Absent
Flavonoids (μg/g)	230.67 ± 0.32
Phenolics (μg/g)	339.83 ± 2.54
Saturated fatty acid (GC-FID)	95.2% (seven types)
Saturated fatty acid (GC-FID)	3.27% (six types)
Mimosine content (HPLC)	0.007% w/w (RSD = 0.41)

HPLC – High-performance liquid chromatographic; FID – Flame ionization detector; GC – Gas chromatography; RSD – Relative standard deviation

Heavy metals

The formulation was incinerated in a silica crucible (Temp: 600°C). The ash obtained was dissolved in Aquaragia and analyzed by Atomic Absorption Spectrometer (Light Source: Hallow cathode lamp; Flame: Air/Acetylene) [Table 1].

Preclinical toxicity evaluation

Preclinical toxicity was performed on Wistar rats according to OECD protocol (for 14 days).[2] Histopathological studies were carried out [Figure 1a-h].

Analysis of fatty acid constituents

Fatty acid analysis of formulation was performed by gas chromatography/flame ionization detector GC/flame ionization detector (FID) equipped with autosampler (Perkin Elmer Clarus 500; Column: Supelcowa × 10, 30 m × 0.25 mm; film thickness 0.25 μm; Carrier gas: H_2; 10 *psi*; Detector: FID; detector temp: 290°C; oven temperature: 130°C for 5 min and raised @ 4°C/min to final temperature of 240°C for 12.5 min; Injector temperature: 260°C; Injection volume: 1.5 μL) [Figure 2a].

Analysis of mimosine in Lajjalu Keram

Mimosine was analyzed using a reversed-phase column chromatography (Shimadzu HPLC with quaternary LC-10A VP pumps; C18 reversed phase Phenomenex column; ultraviolet (UV)-visible detector, Class-VP 5.032 software). The mobile phase was water: Orthophosphoric acid (98.8:0.2, v/v; pH 3.0). Mimosine was eluted isocratically with a flow rate of 1.0 mL min^{-1}. The eluate mimosine was monitored by UV detector at a wavelength of 284 nm).[3] The content of mimosine in the formulation was analyzed [Table 1 and Figure 2b].

Anti-inflammatory activity

Anti-inflammatory activity was carried out against carrageenan-induced rat paw edema as per the method described by Balian using digital plethysmometer.[4] Paw volume was recorded immediately before carrageenan injection and regarded as initial paw volume. Further, paw volume was recorded at the durations of 1, 2, 4, 6, 12, and 24 h after carrageenan injection [Figure 1i]. Percentage inhibition of edema was calculated by using the following formula:

$$\%\,\text{Inhibition at X hours} = \frac{\text{Final volume of Paw (X hours)} - \text{Initial Paw volume}}{\text{Initial Paw volume}} \times 100$$

Shelf life determination

Shelf life of Lajjalu Keram was determined by assaying the content of mimosine at different time intervals up to 6 months after storing the drug on shelf at room temperature. Other organoleptic properties (physical appearance, color, odor, and taste), acid value, viscosity and GC fingerprint were also analyzed [Table 2]. The assay data were further evaluated using Sigmaplot™ 11 (Cranes software International, Bangalore, India) to calculate the shelf life of Lajjalu Keram.

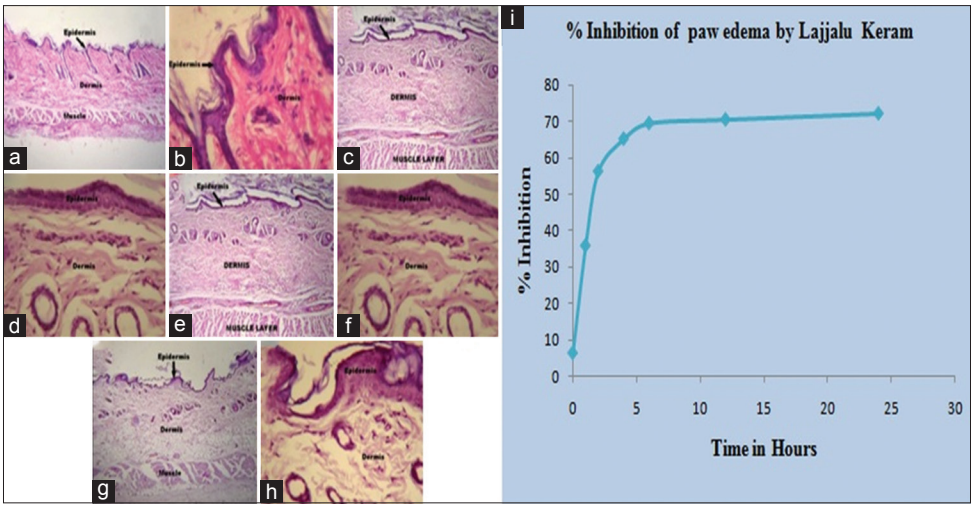

Figure 1: Photomicrograph of sections of skin of different groups showing a normal histological appearance of epidermis and dermis at low resolution (×100) and high resolution (×400). (a) control female group (×100); (b) control female group (×400); (c) control male group (×100); (d) control male group (×400); (e) female rat group treated with Lajjalu Keram (×100); (f). female rat group treated with Lajjalu Keram (×400); (g) male rat group treated with Lajjalu Keram (×100); (h) male rat group treated with Lajjalu Keram (×400). (i) Anti-inflammatory activity of Lajjalu Keram against carrageenan-induced paw edema showing percentage (%) inhibition of paw edema

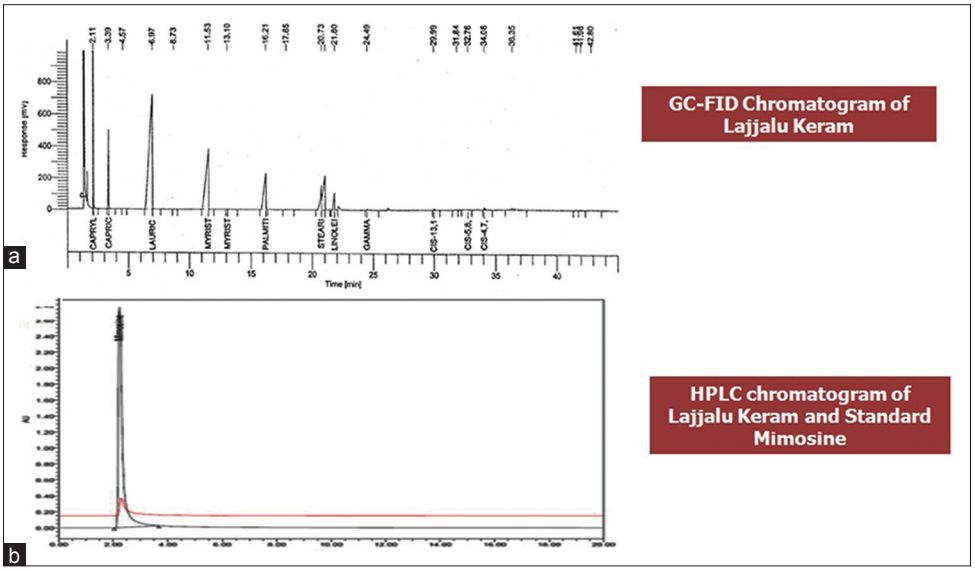

Figure 2: (a) Gas chromatography-flame ionization detector chromatogram showing different types of fatty acids present in Lajjalu Keram (b): Overlay high-performance liquid chromatographic chromatograms showing the presence of mimosine in Lajjalu Keram coinciding with peak of standard mimosine

Results and Discussion

The Lajjalu Keram was studied for standardization as per WHO and AYUSH guidelines. The results of phytochemical analysis confirm the presence of alkaloids, saponin, lipids, phenolic, tannins, and carbohydrate and flavonoid compounds. In this study, absence of total bacterial count has been observed. However, there was presence of colonies forming units with the fungus media. The important finding including heavy metals, aflatoxins, and pesticides were determined to ensure the safety of the drug intended for human use and were found that all are within limit. The finding can be further used as QC tool for formulation evaluation.

Preclinical toxicity studies revealed the safety potential of the formulation because no mortality was observed in the treated (up to 5000 mg kg^{-1} of BW) and control group of animals. No visible signs of treatment (i.e., changes in respiration, circulatory, nervous system, and behavioral

Table 2: Stability studies of Lajjalu Keram at 0, 3, and 6 months for determination of shelf life

Tests	0 month	3 months	6 months
Physical appearance	Hazy liquid	Hazy liquid	Hazy liquid
Color	Greenish	Greenish	Greenish
Odor	Smell of coconut oil	Smell of coconut oil	Smell of coconut oil
Taste	Bitter	Bitter	Bitter
Acid value	1.87±0.23	1.56±0.26	1.13±0.08
Viscosity	56.28±1.35	55.28±1.89	56.28±1.56
GC-FID	13 fatty acids	13 fatty acids	12 fatty acids
Assay of marker	98.36±1.96	98.16±1.65	97.95±1.30

FID – Flame ionization detector; GC – Gas chromatography

pattern) were observed. Necropsy examination did not expose abnormal lesion in any group.

Analysis of fatty acids confirms the presence of fatty acids (total: 98.47%). There were 13 fatty acids detected in the formulation, out of which, seven were saturated fatty acids (95.2%) and the rest were unsaturated fatty acids (3.27%). Among unsaturated fatty acids, maximum amount was of three types of dienoic fatty acid (2.53%).

An rapid HPLC method for quantification of mimosine in the Lajjalu Keram has been developed and validated for the first time for quality control of Lajjalu Keram. The developed and validated method was applied for the analysis of mimosine in Lajjalu Keram. The mimosine content in Lajjalu Keram was found to be 0.0070% w/w with % relative standard deviation of 0.41. The method can be used for routine quality control analysis of Lajjalu Keram because of wide range of linearity, simple mobile phase, UV detection, lack of extraction procedure, low RT, and without using any internal standard.

The formulation afforded significant protection of carrageenan-induced rat paw edema (72.11%) as compared to control. The shelf life studies on antipsoriatic herbal formulations were carried out for 6 months. The assay of constituents using mean curve of sigma plot showed 44.2 months of shelf life Lajjalu Keram.

Conclusion

In brief, the quality control assessment and chromatographical analysis of Lajjalu Keram formulation was performed. The studied formulation was found safe since the absence of pesticides and limit presence of heavy metals and aflatoxins. As no mortality was observed during the study, it can be revealed that the extract could be used as a potential antipsoriatic agent.

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Conflicts of interest
There are no conflicts of interest.

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