



## **Preparation and quality control of a Siddha formulation – Kesari Lehyam**

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### **Abstract**

Any medicine to have international acceptance should have unequivocal proof of its safety, efficacy and quality. In many cases the efficacy and quality are interrelated. Hence, utmost importance needs to be given to prove the quality of herbal drugs. The proposed work involves the development and validation of the selected Siddha formulation – KESARI LEHYAM (KL) by determining various Physicochemical & Phytochemical parameters. The individual drugs were subjected to Organoleptical/Morphological screening in order to ascertain their authenticity. KL was subjected to systematic Phytochemical screening in order to ascertain various Phytochemical constituents present in KL formulation. HPTLC fingerprint profiles for the extracts were established and co-chromatography was carried out by using marker compound, piperine. For establishing fingerprint profiles, the methanolic and ethyl acetate extracts of Standard Formulation and Commercial Formulation of Kesari Lehyam (KL) were resolved by using the selected solvent system. The finger print profile of the chromatogram of various extracts can be considered as a reference to characterize the presence of other constituents in the formulation of KL. The research findings will help to provide quality product to the consumers and to expand the market for the herbal products to international level and thereby we can popularise our Indigenous Medicine globally.

**Keywords:** Kesari Lehyam (KL), HPTLC Analysis, Standard Formulation (SF), Commercial Formulation (CF)

### **Introduction**

Some of the plants are known to contain characterizing compounds that are specific to the species or family. These characterizing compounds are referred to as the chemical marker compounds which are biologically or therapeutically active principles. The plant material can be standardized with these markers and quantified-in-the-plant. In case of processed plant materials the quantification is done with the changes in the quantities of these marker compounds. Normally these chemical marker compounds are available amongst alkaloidal and some types of glycosidic crude drugs. Majority of chemical composition of higher plants in general is common to

all plants and we need to direct our efforts to develop assay procedures for such type of components.[Saraswathy A (2003)] .

Commercially, our country has lagged far behind in the international market for traditional drugs. The use of genuine and authentic plant material is essential for production of quality drugs in these systems of medicine. In fact credibility of these systems depends upon the availability and authenticity of crude drugs [Bhutani K.K. (2003)]. One of the best methods of standardizing herbs and herbal formulations based on modern scientific tools is chromatography. It not only

helps in establishing the correct botanical identity but also helps in regulating the sanctity of the herb. Keeping in view the importance of standardization of herbal drugs, the present study was carried out.

## Materials and Methods

All the herbal drugs including fresh Lemon fruits (Normal size) were purchased from the local Crude Drugs seller shop (Naattu Marunthu) in Villuppuram, Tamil Nadu and the drugs were authenticated by the Professor of Pharmacognosy, Department of Pharmacy, Annamalai University, Annamalai Nagar, Tamil Nadu. The standard drug pure piperine compound was purchased from Sigma (Aldrich), Bangaluru, Karnataka and assigned purity: 99%. The marketed Kesari Lehyam was purchased from M/S IMCOPS, Adyar, Chennai. The vanillin reagent used for visualization was from Merck (Germany), and the solvents (toluene and ethyl acetate) were from Sigma (Aldrich). All chemicals used were of analytical grade. The vanillin sulphuric acid agent was prepared accordance with standard Text Book.

### Preparation of Kesari Lehyam formulation [The Siddha Formulary of India, (1986)]:

Ingredients:	Qty
1.Lemon fruit (Normal size)	:50Nos
2.Milagu ( <i>Piper nigrum</i> )	: 50G
3.Daniya ( <i>C.sativum</i> )	:50G
4.Koshtam ( <i>Costus speciosus</i> )	:05G
5.Chukku (Dried Ginger)	:05G
6.Thaleesapatri (( <i>Taxus baccata</i> )	: 05G
7.Tippili ( <i>Piper longum</i> )	:05G
8.Jathikkai ( <i>Myrisica fragrans</i> )	:05G
9.Jathipathri (Mace)	:05G
10.Ghee	:120G
11.Sugar	: Quantity sufficient

### Preparation:

The lemon fruits were fried in an earthen pan and the juice was extracted. To the extracted juice twice the amount of sugar was added and boiled till it attains pakam (a semisolid form). The weighed amount of drugs in powdered form (No.2 to 9) were added one by one slowly mixing well till it attains Lehya pakam. The Ghee was added finally and mixed well and the resulting Lehyam was stored in a clean container for further analysis.

### Examination of the Organoleptical/Morphological characteristics of the Drugs [Kokate,C.K,et al, (1997)]:

In macroscopical study, size, shape, colour, odour and taste of the purchased ingredients were examined to ascertain their authenticity.

### Phyto chemical Screening [Handa S.S et al, (2001)].

The general qualitative phyto chemical screening is based on a color reaction or precipitation in response to a particular reagent. In order to test the phyto constituents of a specific chemical class an appropriate extraction procedure has to be adopted. Extracts may also need to be concentrated for a visible reaction to take place. Many of the chemical tests given here are applied as identification tests in Pharmacopoeial monographs for crude drugs known to contain those constituents and can be adopted for use as TLC spraying agents or to produce colored solutions for quantitative analysis. Reference materials are preferable on which the tests may be performed (in parallel with the sample ) as positive controls. The results of these tests must be taken in conjunction with those of other analytical methods before final conclusions can be drawn. Three extracts – viz- chloroform, alcoholic and water extracts were prepared for the Kesari Lehyam sample by using respective solvents.

### Chromatographic finger prints analysis by HPTLC [Sheetal Gawas et al, (1999)].

Fingerprint analysis by HPTLC or HPLC is one of the most powerful tools to link the botanical identity to the chemical constituent profile of the plant. In combination with microscopic investigations, the fingerprint provides the means for a convenient identity check. It can also be used to detect adulterations in raw materials. From the constituent profile, a number of marker compounds can be chosen which might be used to further describe the quality of the herb or the herbal preparation. High performance thin layer chromatography can also be employed for quantitative determination of such marker compounds. Some of the plants are known to contain characterizing compounds that are specific to the species or family. These characterizing compounds are referred to as the chemical marker compounds which are biologically or therapeutically active principles. The plant material can be standardized with these markers and quantified-in-the-plant. In case of processed plant materials the quantification is done with the changes in the quantities of these marker

compounds. Normally these chemical marker compounds are available amongst alkaloidal and some types of glycosidic crude drugs. Majority of chemical composition of higher plants in general is common to all plants and we need to direct our efforts to develop assay procedures for such type of components [Pulok K. Mukherjee. (2001)].

Commercially, our country has lagged far behind in the international market for traditional drugs. The use of genuine and authentic plant material is essential for production of quality drugs in these systems of medicine. In fact credibility of these systems depends upon the availability and authenticity of crude drugs. Chromatography, the best method of standardizing herbs and herbal formulations, not only helps in establishing the correct botanical identity but also helps in regulating the sanctity of the herb.

### Sample Preparation

About 5 g of the air dried sample drug was extracted with 100 ml of 90% ethanol in a closed flask for 24 hours shaking frequently during first six hours and allowing to stand for 18 hours. It was filtered rapidly, taking precautions against loss of alcohol. The filtrate was evaporated at a low temperature in a flat-bottomed flask and transferred to a volumetric flask and made up to 10 ml.

### Preparation of standard solutions

#### Piperine Solution.

A stock standard solution of piperine (1mg/ml) was prepared by dissolving 10 mg of accurately weighed piperine in methanol and making up the volume to 10ml with more methanol in amber coloured volumetric flask covered with aluminium foil. The stock solution was further diluted with methanol to give a standard solution of piperine (10 ng/ml). Another standard solution of piperine was prepared with ethyl acetate solvent in the same manner. Two standard solutions with different solvents were prepared to ascertain the type of solvent best suitable to produce a single sharp peak for piperine (Eapen Saumy et al., 2002).

#### Mobile phase

Toluene: Ethyl Acetate in the ratio 7:3 v/v.

### Procedure:

The samples were spotted in the form of bands of width 3mm with a Camag micro litre syringe on pre-coated silica gel aluminium plate 60 F254 (10 cm × 10 cm with 0.2 mm thickness; E. Merck, Darmstad, Germany) using a Camag Linomat V (CAMAG, Muttenz, Switzerland). A constant application rate of 150 nL/s was employed and space between two bands was 4 mm. The slit dimension was kept at 4 mm × 0.1 mm, and 20 mm/s scanning speed was employed. These parameters were kept constant throughout the analysis of samples. The mobile phase consisted of toluene and ethyl acetate in a ratio of 7: 3 v/v. Plates were developed in ascending order with a CAMAG twin trough glass tank which was pre-saturated with the mobile phase for 15 min; the length of each run was 8 cm. The TLC runs were performed under laboratory conditions of (Temp: 25 ± 2°C and % RH: 60 ± 5). The plates were then dried in air. Densitometric analysis was performed at 513 nm with a Camag TLC scanner III operated by Win CATS software (Version 1.2.0). The source of radiation utilized was deuterium and tungsten lamp. The composition of the mobile phase for TLC was optimized using different solvents of varying polarity and good resolution was achieved using toluene: ethyl acetate (7: 3 v/v) as mobile phase.

The Finger print profile of the samples developed in the HPTLC plate was photographed after spraying with vanillin - sulphuric acid agent.

### Results

#### Morphological Screening:

The individual drugs were subjected to Organoleptical/ Morphological screening in order to ascertain their authenticity. All the crude drugs namely Milagu (*Piper nigrum*), Daniya (*C.sativum*), Koshtam (*Costus speciosus*), Chukku (Dried Ginger – *Zingiber officinalis*), Thaleesapatri (*Taxus baccata*), Tippili (*Piper longum*), Jathikkai (*Myristica fragrans*), and Jathipatri (Mace - *Myristica fragrans*) were shown the characters as described in the Literature above.

#### Phytochemical Screening:

The Kesari Lehyam sample showed the presence of Alkaloids, Glcosides, Flavonoids, Tannins & phenolic compounds, Carbohydrates and Volatile oils in the chloroform extract; Alkaloids, Glcosides, Flavonoids, Tannins & phenolic compounds, Carbohydrates, Proteins and free amino acids and Volatile oils in the

alcoholic extract while Aqueous extract showed the presence of Glcosides, Flavonoids, Tannins & phenolic compounds, Carbohydrates, Proteins and free

amino acids and Carbohydrates. The results are given below.

Phytochemical Screening	Chloroform extract	Alcohol extract	Aqueous extract
Alkaloids	+	+	-
Glycosides	+	+	+
Steroids,	-	-	+
Flavonoids	+	+	+
Tannins & phenolic compounds	+	+	+
Proteins and free amino acids	-	+	+
Carbohydrates	+	+	+
Volatile oils	+	+	-
Saponins	-	-	-

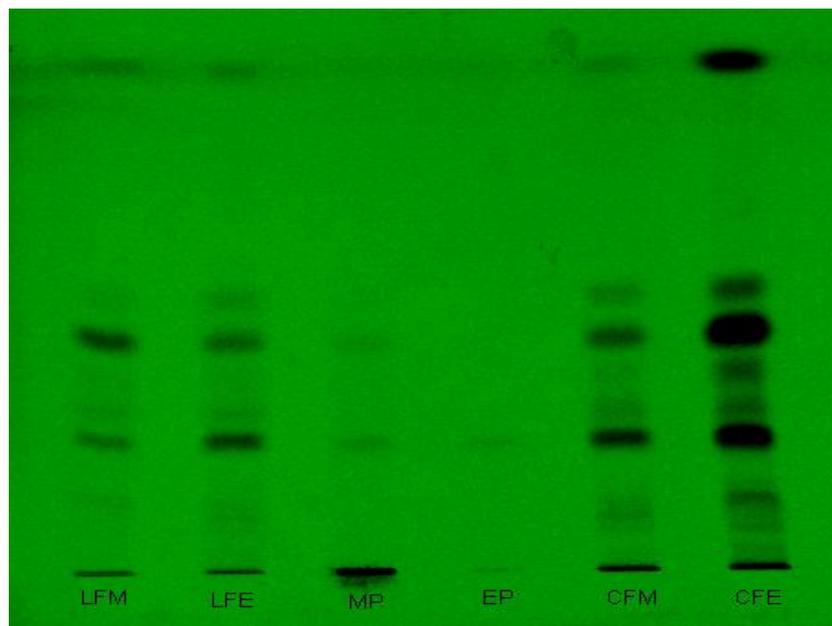
### Results of HPTLC Analysis

Using the extracts, preliminary study was carried out to select suitable mobile phase, which can separate the marker compound from sample matrix by using various solvents. Aliquot portions of standard solutions of piperine and KL (Kesari Lehyam) extracts were applied on a TLC plate for producing a finger print profile apart from determining the presence of piperine in KL, in the form of 6 mm bands with help of CAMAG Linomat V. Different solvents with varying polarity as well as combination of solvents were tried to get well separated bands of the

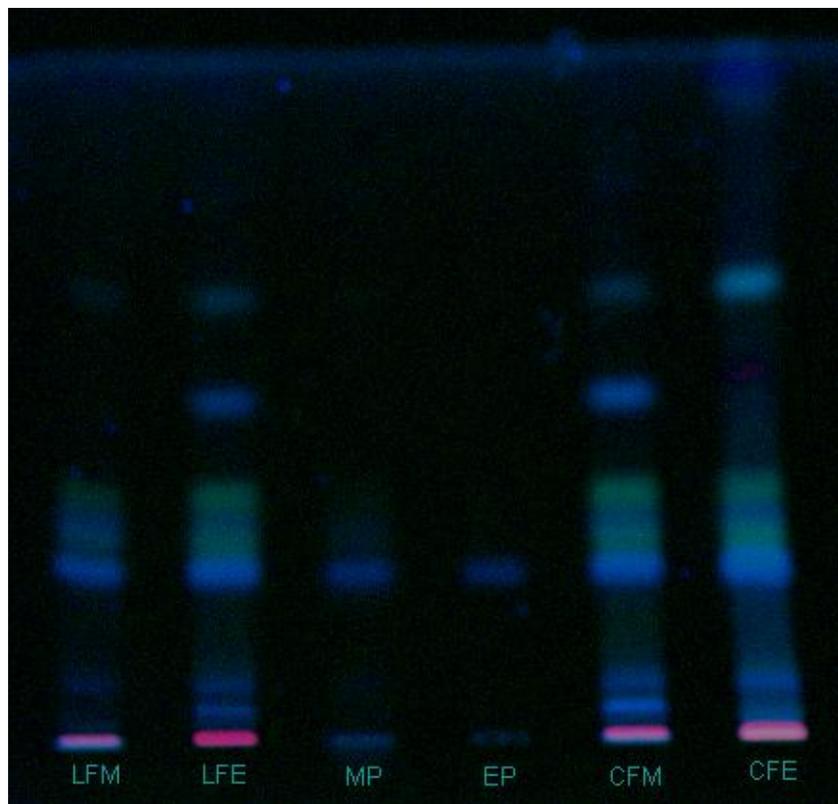
drugs. After trying several combinations, the solvent system containing Toluene : Ethyl acetate in the ratio 7:3 v/v was found to be most satisfactory solvent system as it gave good resolution for the above marker compound. [Plate I and Plate II]

The spot at Rf: 0.26 corresponds to piperine was observed in the respective chromatograms of the extracts of SF and CF. There was no interference from other components present in the formulation as indicated by the shape of the spectrum of the respective spots.

Plate I scanned at 254 nm (KL)



- LFM** – Standard formulation - methanol extract of KL
- LFE** – Standard formulation - ethyl acetate extract.
- MP** – Standard piperine in methanol
- EP** – Standard piperine in ethyl acetate
- CFM** – Commercial formulation - methanol extract of KL.
- CFE** – Commercial formulation - ethyl acetate extract of KL.



- LFM** – Standard formulation - methanol extract of KL
- LFE** – Standard formulation - ethyl acetate extract of KL.
- MP** – Standard piperine in methanol
- EP** – Standard piperine in ethyl acetate
- CFM** – Commercial formulation - methanol extract of KL.
- CFE** – Commercial formulation - ethyl acetate extract of KL.

## Discussion

TLC fingerprint profiles were established and co-chromatography was carried out using marker compound, piperine. For establishing fingerprint profiles, the methanolic and ethyl acetate extracts of SF and CF of KL were resolved by using the selected solvent system which was described in the estimation of marker compounds. Chromatogram and absorption spectra of the resolved bands were recorded. The relative percentage of the resolved bands also was noted. When scanned at 254 nm the methanolic and ethyl acetate extracts of SF produced eight bands each while CF produced eight and seven bands respectively, of which one band at corresponding to Rf value of 0.26 corresponds to piperine. From this profile, it is revealed that all the samples of KL contain piperine. From the above study, it is revealed that ethyl acetate is a better solvent to extract the marker compound piperine from SF and CF of KL. It is also revealed that methanol also can extract piperine

to a considerable extent but not to the extent of ethyl acetate. Purity of the band of the marker was confirmed by overlaying the absorption spectra recorded at start, middle and end position of the band.

The reasons for selecting only piperine as the marker compound, from KL formulation are due to the non availability of other compounds at an economical price. However other marker compounds can be included for future studies.

The finger print profile of the chromatogram of various extracts can be considered as a reference to characterize the presence of other constituents in the formulation of KL. The TLC fingerprint of the sample under test may be compared with the TLC fingerprint of the formulation reference standard believed to be genuine material and of good quality. Thus, availability of this formulation reference standard becomes very crucial for quality control of marketed KL formulations.

## Conclusion

A standardized product can ensure that sufficient amounts of the herb's constituents are present to deliver an efficacious product. Keeping in view the importance of standardization of herbal drugs, the present study was carried out. The finger print profile of the chromatogram of various extracts can be considered as a reference to characterize the presence of other constituents in the formulation of KL. In order to make validity of the quality control of the Kesari Lehyam formulation more stringent, quantitative estimation of some more marker compounds present in the formulation is very essential.

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