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Research Article

Development and validation of a RP-HPLC method for quantification of rottlerin in Kamala (*Mallotus philppinensis*)

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ABSTRACT

Kamala is orange red powder consisting the glands and hair covering the fruit of *Mallotus philppinensis* Muell. (Euphorbiaceae), a small tree widely distributed throughout India up to an attitude of 1500 m. It is indigenous to southeastern Asia and find wild throughout tropical Asia, Australia and Philippines. In the indigenous system of medicine, Kamala is used as anthelminitic and cathartic. Kamala powder is used in external application for parasitic affection of the skin and also an aphrodisiac, lithotropic, and stypic. Kamala powder is also used as an antiseptic in blisters in the ears. The seed and fruit powder are generally used for urinary and menstrual disorders. A selective, precise and accurate Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method has been developed for the quantification of Rottlerin in kamala. The use of reverse phase C18 column using gradient mobile phase of acetonitrile and water enabled the efficient separation of the chemical markers within a 22 minute analysis. Validation of method performs in order to demonstrate its selectivity, accuracy, precision, repeatability and recovery study. The proposed RP-HPLC method was found to be simple, precise and accurate and can be used for the quality control of the raw materials as well as formulations.

Keywords: Kamala, *Mallotus philppinensis*, Rottlerin, RP-HPLC.

INTRODUCTION

Kamala (Kampillaka) is well described in *Charaka Samhita* [1] and *Sushruta samhita* [2]. The glands and hairs of the fruits are used to remove intestinal worms and also as a purgative. It is stated to be undoubtfully effective in intestinal worms when administered with jaggery [3].

Kamala (*Mallotus philippinensis* Muell.) belongs to the family Euphorbiaceae. The tree grows throughout tropical India and particularly along the foot of Himalaya from Kashmir Eastwards upto a height of 1500 m [4,5]. During the month of February-March its fruits ripen becoming brick-red in colour [6]. The mature fruits are collected and the hairs and glands are gently separated from them.

Traditionally Kamala is used as an anthelminitic. Rottlerin and Isorottlerin are responsible for the anthelminitic activity of the Kamala [7]. Kamala powder is used in external application for parasitic affection of the skin and also an aphrodisiac, lithotropic, and stypic. Kamala powder is also used as an antiseptic in blisters in the ears. The seed and fruit powder are generally used for urinary and menstrual disorders [8,9].

The samples of kamala were subjected to chemical analysis with particular reference to Rottlerin through Reverse Phase High Performance Liquid Chromatography (RP-HPLC).

MATERIALS AND METHODS:

The kamala were collected from local market in Ahmadabad city, Gujarat, India and authenticated by comparison with herbarium specimens [10]. Rottlerin was isolated as per the method described by Khorana and Motilal [11]. HPLC grade acetonitrile, water and methanol were obtained from Merck (Darmstadt, Germany).

Instrumentation:

Analysis were performed on a HPLC of Shimadzu LC-20AD model equipped with an online degaser DGU-20 As, a Rheodyne 7725 injection valve furnished with 20 µl loop, a SPD-M20A photodiode

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Table 1: Quantification of Rottlerin in kamala by HPLC method.

Sample	Amount of Rottlerin[a] (% w/w)
Kamala powder	21.251 ± 0.11

[a] Mean ± SD (n=6)

Table 2: Regression parameter, Linearity, Limit of Detection (LOD) and Limit of Quantification (LOQ) of the proposed HPLC method.

Compound	Concentration range (µg/ml)	Rt (min)[a]	Regression equation	R^2	LOD	LOQ
Rottlerin	10-60	17.883 ± 0.03	y = 39764x + 91252	0.996	0.45	1.9

[a] Mean ± SD (n=6)

Table 3: Repeatability and Recovery tests for the marker in Kamala.

Compound	Contents[a] (mg/g)	Added amount (mg)	Recorded amount[a] (mg)	Recovery rate[a] (%)	RSD (%)
Rottlerin	212.51 ± 0.11	100	313.54 ± 1.07	100.33 ± 0.73	0.73
		200	406.53 ± 2.04	98.55 ± 1.55	1.57
		300	570.90 ± 1.08	99.10 ± 1.83	1.84

[a] Mean ± SD (n=3)

Table 4: Precision of the Intra-daily and Inter-daily HPLC measurement for marker in Kamala.

Compound	Intra-daily[b]		Inter-daily[c]	
	Contents[a] (% w/w)	RSD (%)	Contents[a] (% w/w)	RSD (%)
Rottlerin	21.251 ± 0.11	1.81	21.219 ± 0.10	0.22

[a] Mean ± SD (n=6)
[b] Samples was analyzed six times a day
[c] Sampls was analyzed once a day over six consecutive days

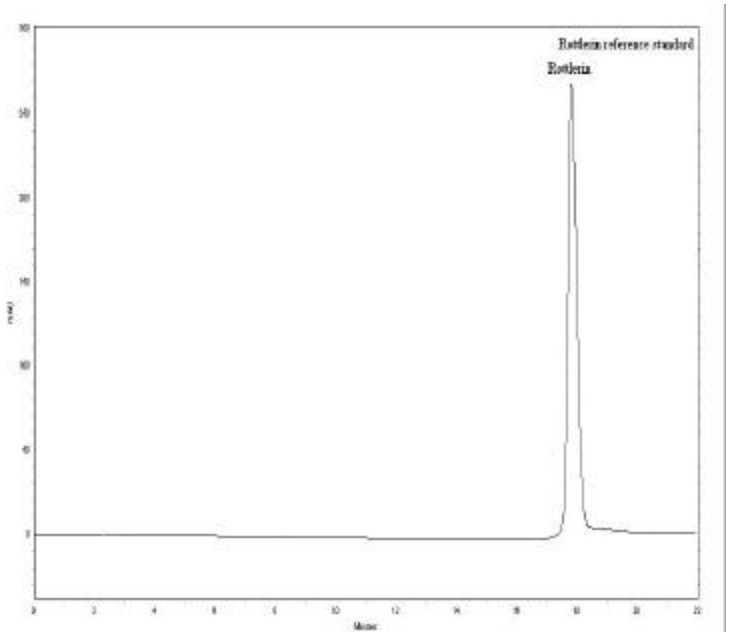

Figure 1: RP-HPLC Chromatogram of Rottlerin

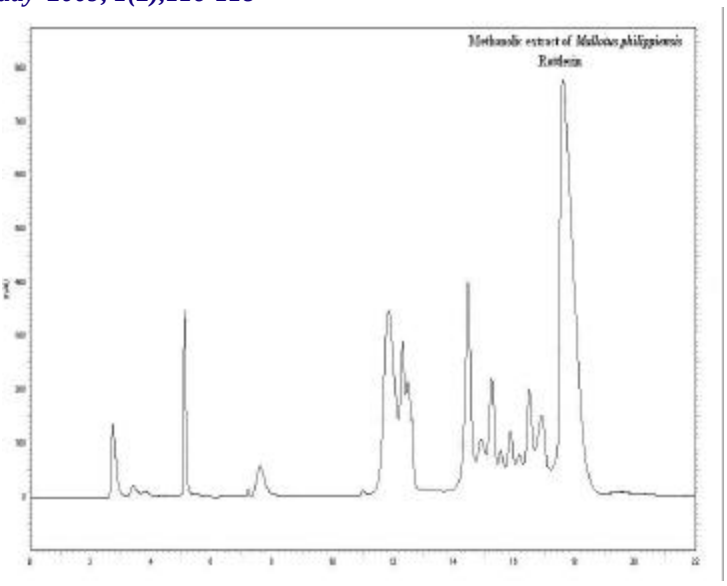

Figure 2: RP-HPLC Chromatogram of Kamala

array detector and a Class-VP software. Separation was carried out using a Phenomenax column (250 × 4.6 mm i.d., 5 µm pore size). The column was maintained at 27° C throughout analysis and detection was carried at 298 nm.

Sample preparation:

A 100 mg powdered Kamala was extracted three times with 100 ml methanol. The extract were combined and concentrated at reduce temperature (50° C) on rotary evaporator (Equitron rotevar, Medica instrument mfg. co.) upto 100 ml. Prior to use, sample were filtered through a 0.45 µm nylon membrane filter.

Calibration:

The content of the marker was determined using a calibration curve established with six dilutions of standard, at concentrations ranging from 10 to 60 µg/ml. Each concentration was measured in triplicate. The corresponding peak areas were plotted against the concentration of the marker injected. Peak identification was achieved by comparison of both the retention time (Rt) and UV absorption spectrum with those obtained for standard. The calibration curves of the reference Rottlerin employed was shown in Fig. 1.

Validation parameter:

The method was validated according to ICH guideline for linearity, precision, accuracy, selectivity, limit of detection and limit of quantification [12].

Selectivity was checked using an extract of kamala and a standard in order to optimize separation and detection. Linearity of the method was performed by analyzing a standard solution of marker by the proposed method in the concentration range 10-60 µg/ml. The accuracy of the proposed method was determined by a recovery study, while was carried out by adding standard marker in the kamala extract.

The samples were spiked with three different amounts of standard compound prior to extraction. The spiked sample was extracted in triplicate and analyzed under the previously established optimal conditions. The obtained average content of the target compound was used as the actual values in order to calculate the spike recoveries. Precision was determined by repeatability and interday and intraday reproducibility experiments of the proposed method. A standard solution containing 3 markers was injected 6 times, kamala was also extracted 6 times to evaluate the repeatability of the extraction process. The mean amounts and SD value of each constitute were calculated.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The LOD and LOQ of markers compounds were calculated at signal-to-noise ratio of approximate 3:1 and 10:1 respectively.

RESULT AND DISCUSSION:

Optimization of HPLC Chromatographic Conditions:

Optimum chromatographic conditions were obtained after running different mobile phase with a reverse phase C18 column. Acetonitrile was preferred over methanol as mobile phase because its use resulted in improved separation. Many different gradient systems of mobile phase were tried for the best separation of peaks. Selecting 298 nm as the detection wavelength resulted in an acceptable responses and enable the detection of marker used in this study. The column was used at 27^{o}C. An HPLC fingerprint for the kamala was developed. Elution was carried out at a flow rate of 0.5 ml/min with acetonitrile as solvent A and water as solvent B using gradients elution in 0-10 min with 50-40 % A, 10-13 min with 40-35 % A, 13-17 min with 35-30 % A and 17-22 min with 30-10 % A. Each run was followed by a 10 min wash with 10 % acetonitrile and an equilibration period of 15 min.

Quantification of marker present in kamala:

The marker that was used for quantification was found in kamala (Fig. 2). The chromatogram of kamala was quantified with respect to Rottlerin (21.251 % w/w).

Method validation for HPLC fingerprinting method:

The HPLC method was validated by defining the selectivity, linearity, accuracy, precision, limits of detection and limit of quantification. For qualitative purposes, the method was evaluated by taking into account the precision in the retention time and selectivity of marker compounds eluted. A high repeatability in the retention time was obtained both standards and extracts even at high concentration. For quantitative purpose linearity, accuracy, precision LOD and LOQ were evaluated. LOD and LOQ values for Rottlerin 0.45 µg/ml and 1.9 µg/ml respectively. Linear correlation was obtained between peak area and concentration of marker in the range of 10-60 µg/ml. Values of the regression coefficients (r^2) of the marker was higher than 0.99, thus confirming the linearity of the method (Table 2). The high recovery values (98.55-102.33 %) indicated a satisfactory accuracy. Relative standard deviation was less than 2.24 % for the degree of repeatability, indicating the high repeatability of the proposed methods. The low coefficient of variation values of intraday and interday precision reveals that the proposed method is precise (Table 4). Therefore, this HPLC method can be regarded as selective, accurate and precise.

CONCLUSION:

The results indicate that kamala contains a number of markers that may be responsible for the therapeutic activity. The HPLC method developed will assist in the standardization of kamala using biologically active chemical marker. Kamala also contained a number of other constitute, which are currently the subject of further investigation, apart from these standard studied. With the growing demand for herbal drugs and with increased belief in the usage of herbal medicine, this standardization tool will help in maintaining the quality and batch to batch consistency of this important medicinal plant.

REFERENCES:

1. Agnivesha. Kampillaka, Charaka Samhita. 5th ed. Chowkambha Sanskrit Sansthan: Varanasi; 2001.
2. Sushruta. Kampillaka, Sushruta Samhita. 4h ed. Chowkambha Orientalia: Varanasi; 1980.
3. Bhavamishra. Krimichikitsa, Bhavaprakasha. 7th ed. Chaukambha Sanskrit Sansthan: Varanasi; 2000.
4. Warrier PK. Mallotus philippinensis Muell. Arg, in Indian Medical Plants. Vol. III. Orient Longman Ltd: Madras, India; 1995, 375.
5. Hooker JD. Mallotus philippinensis Muell. Arg, In the Flora of British India, Vol. V. Under the authority of the secretary of state for India in Council: India; 1890, 443.
6. Anonymous. The Wealth of India: Raw Materials. Centre council for Scientific and Industrial Research: New Delhi; 1984, 230.
7. Anonymous. The Wealth of India: Raw Materials. Vol.-IV. Centre Council for Scientific and Industrial Research: New Delhi; 1952, 229.
8. Nadkarni KM, Nadkarni AK. Indian Materia Medica. 3rd ed. Vol.-I. Popular Prakashan: Bombay; 1979, 760.
9. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. Centre Council for Scientific and Industrial Research: New Delhi; 1956, 160.
10. Mukherjee PK. Quality Control of Herbal Drugs. Business Horizons: New Delhi, India; 2005, 741-743.
11. Khorana ML & Motiwala DK. Indian J. Pharm., 1949; 11: 37-43.
12. Anonymous. Guideline on Validation of Analytical Procedure-Methodology. International Conference on Harmonization: Geneva, Switzerland; 1996.

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