ORIGINAL ARTICLE

Pharmacognostic evaluation of an ayurvedic formulation *Eladi Gutika*

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Eladi Gutika (*EG*), a traditional Ayurvedic polyherbal formulation is used as a remedy for *Kasa* (Cough), *Svasa* (Asthma), *Bhrama* (Vertigo), *Raktapitta* (Bleeding disorders), *Jvara* (Fever), *Amvata* (Rheumatism) etc. In the present work, an attempt has been made to develop pharmacognostic standards for *EG*. The raw materials of *EG* were subjected to proximate analysis prior to preparation of *EG*. *EG* was prepared using raw materials of pharmacopoeial quality. Powder microscopy of *EG* showed the presence of discerning anatomical characters which were present in the raw materials. Preliminary phytochemical evaluation of *EG* revealed the presence of carbohydrates, alkaloids, flavonoids, essential oils, glycosides and tannins. A simple, rapid, accurate and sensitive HPTLC method was developed and validated for the quantitation of piperine from *EG*. Method was validated as per ICH guidelines and applied for stability studies of *EG* stored for different storage periods at room temperature. A comparative evaluation of *EG* prepared in-house was carried out with three available marketed samples in terms of their respective piperine content. Acute toxicity study of *EG* in Albino Swiss mice revealed that it is safe at the dose of 2 g/kg body weight in animal. Evaluation of *EG* by these scientific methods can be used as quality control tool for the manufacturing and processing of *EG*.

Key words: *Eladi gutika*, HPTLC, piperine, quality control, standardization

INTRODUCTION

Modern analytical techniques like HPTLC, HPLC, FTIR etc have been routinely used for assessing the quality of the raw material and many traditional formulations.[1-4]

Eladi Gutika (EG), is an Ayurvedic polyherbal formulation of seven ingredients namely *Ela* (*Ellettaria cardamomum*), *Tejpatra* (*Cinnamomum tamala*), *Tvak* (*Cinnamomum verum*), *Pippali* (*Piper longum*), *Madhuka* (*Glycirrhiza glabra*), *Kharjura* (*Phoenix sylvestris*), *Mrdvika* (*Vitis vinifera*), *Sita* (Sugar) and *Madhu* (Honey).[5] It is prescribed for the treatment of cough and asthma.[5]

Due to lack of modern pharmacopoeial standards laid down and followed for processing of *EG*, the medicine may not have desired quality and batch to batch consistency. Hence, there is a need for standardization of *EG* using modern bioanalytical tools.[6]

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In the present work, quality of the raw materials used for preparation of *EG*, was evaluated on the basis of ash (total, acid insoluble and water soluble) content.[7] *EG* (in house) was prepared in the Herbal Research Laboratory of Ramnarain Ruia College, Mumbai as per the classical reference.[5] Preliminary phytochemicals of *EG* were evaluated as per standard methods.[8]

Piperine [Figure 1] a major alkaloid of *Pippali* (one of the ingredient of *EG*) is reported as a bioavailability enhancer, anti-inflammatory, anticonvulsant and antiulcer agent.[9-11] There are reports on extraction of piperine using various extraction techniques and its estimation using analytical tools from single herbs and polyherbal formulations.[3,4,12,13] But, there are no methods reported for its estimation from complex matrix of *EG*.

In this report a precise, accurate and reproducible HPTLC densitometric method was developed and validated[14] for quantitation of piperine from the complex matrix of *EG*. Method was applied to evaluate the impact of storage periods on the piperine content of stability samples and also to comparatively evaluate marketed samples of *EG* with in house prepared *EG*. Safety of the *EG* was evaluated by conducting acute toxicity studies in mice.

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MATERIALS AND METHODS

Plant Materials

Raw materials used for the preparation of *EG* and three different marketed brands of *EG* (M-01, M-02, M-03) were procured from Bharat Aushadhi Bhandar, Pydhonie, Mumbai and authenticated by Herbal Research Lab, Ramnarain Ruia College. Collected materials were dried in incubator at 45°C, powdered and sieved through an 85-mesh (BSS) sieve. All the raw materials were stored in air tight containers at room temperature.

Standard and Reagents

The organic solvents and chemicals of analytical grade were procured from Qualigens Fine Chemicals, Mumbai, India. Standard Piperine (≥ 99 % purity, HPLC grade, [Figure 1] was procured from Sigma Aldrich Chemical Company, Germany.

Preparation of *Eladi Gutika*

EG was prepared as per classical reference. Formula composition of *EG* is presented in Table 1. *Kharjura, Mrdvika* and *Madhu* were thoroughly mixed together to form a homogenous mixture to which *Sita* (Sugar) was added and grounded well. To this, mixture of *Madhuka, Ela, Patra, Tvak* and *Pipali* was added and mixed well to form a homogenous blend. Soft spherical pills were made from this blend; shade dried and packed in air tight containers for further analysis.

Proximate Analysis of *Eladi Gutika*

All the raw materials of *EG* were subjected to physicochemical quality check prior to use them for preparation of *EG.* Ash (total ash, acid insoluble ash and water soluble ash) content of *EG* was determined using standard pharmacopoeial methods.[7]

Powder Microscopy

EG and its ingredients were microscopically evaluated to reveal their anatomical characters. Similar discerning characters were observed in *EG* as those seen in ingredients.[15]

Preliminary Phytochemical Evaluation

Preliminary evaluation of phytochemicals of *EG* was carried out by performing tests for flavonoids, essential oils, tannins, glycosides, alkaloids and resins as per standard methods.[8]

Chromatographic Evaluation

HPTLC conditions

Chromatographic separation was achieved on HPTLC plates precoated with silica gel 60 F_{254}(E. Merck) of 0.2 mm thickness with aluminum sheet support. Samples were spotted using CAMAG Linomat IV Automatic Sample Spotter (Camag Muttenz, Switzerland) equipped with

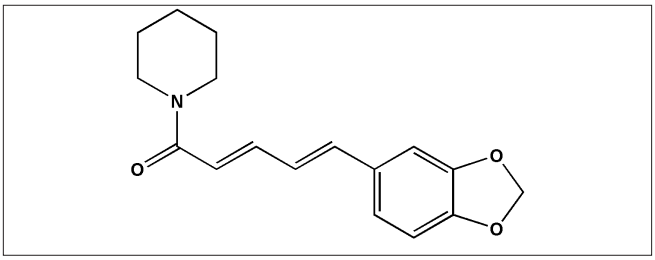

Figure 1: Structure of Piperine

Table 1: Formula composition for *Eladi Gutika*

Ingredients		Quantity (parts)
Ayurvedic name	**Part used**	
Ela	Seeds of *Ellettaria cardamomum*	1 part
Patra	Leaves of *Cinnamomum tamala*	1 part
Tvak	Stem bark of *Cinnamomum verum*	1 part
Pippali	Fruits of *Piper longum*	4 parts
Madhuka	Root of *Glycirrhiza glabra*	8 parts
Kharjura	*Phoenix sylvestris*	8 parts
Mrdvika	Dried fruit of *Vitis vinifera*	8 parts
Sita	Sugar	8 parts
Madhu	Honey	8 parts

syringe (Hamilton, 100 µL). Plates were developed in a glass twin trough chamber (CAMAG) pre-saturated with mobile phase. Scanning device used was CAMAG TLC Scanner II equipped with CATS 3 software. The experimental condition was maintained at 20 ± 2°C. Detection of piperine was possible after derivatizing the plates with anisaldehyde sulphuric acid reagent and photo documentation with CAMAG Reprostar 3 at 550 nm.

Stock Solution of Piperine

Stock solution of piperine (1000 µg mL^{-1}) was prepared by dissolving 10 mg of accurately weighed standard in methanol and making up the volume to 10 mL in standard volumetric flask. Aliquots of 20-80 µg mL^{-1} were prepared from this stock solution for calibration curve. Three quality control (QC) samples viz. low (LQC, 25 µg mL^{-1}), medium (MQC, 40 µg mL^{-1}) and high (HQC, 65 µg mL^{-1}) of piperine were prepared for precision, accuracy and ruggedness studies.

Extraction of Piperine from *Eladi Gutika, Piper longum* and Marketed Samples

Extraction of marker components from *EG* was a daunting task because of its complex polyherbal matrix. Hence, extraction conditions were optimized to achieve good fingerprinting and to resolve piperine efficiently. Different solvents and solvent to sample ratios were tested and finally *EG* (1 g) was extracted with 10 mL of chloroform, vortexed for 1-2 minutes and kept standing overnight at room temperature. The mixture was filtered through Whatmann filter paper No. 41 (E. Merck, Mumbai,

India), evaporated to dryness and reconstituted in 1 ml of methanol. The filtrate was re-filtered and used for HPTLC analysis. Similar extraction procedure was followed for marketed samples of *EG*.

Solvent System

Solvent system consisting of toluene: ethyl acetate: formic acid (6: 3: 0.3, v/v/v) was used to resolve and quantify piperine from the matrix of *EG* and marketed samples.

Method Validation

ICH guidelines were followed for the validation of the developed HPTLC method in terms of specificity, precision, sensitivity, recovery and ruggedness.[14]

Estimation of Piperine from *Eladi Gutika*

Samples (10 µL) of *EG* were applied in triplicate to a pre-coated silica gel 60 F_{254} HPTLC plate (E. Merck) with the Camag Linomat IV sample spotter. The plate was developed and analysed as per the optimized chromatographic condition.

Method Application

The developed HPTLC method was applied further to study the stability of *EG* samples stored at different storage periods. Comparative evaluation of in-house *EG* was carried out with three marketed brands (M-01, M-02 and M-03) in terms of their respective Piperine content using developed method.

Safety Evaluation

As a safety parameter, acute oral toxicity of *EG* was conducted on Albino Swiss mice weighing 18-22 g. Animals were purchased from Hafkins Institute, Mumbai. The animals were grouped in two groups containing three female mice per group. First group received aqueous slurry of *EG* orally at the dose of 2g/kg body weight of the animal while the second group received 2 ml of distilled water. Study was conducted as per the methodology laid down in the OECD guideline 420 viz., Fixed Dose procedure (Evident Toxicity). Toxicity was evaluated in terms of mortality, daily food, water intake, body weight increments and general behavioral changes.[16]

RESULTS AND DISCUSSION

Quality assurance is an integral part of all systems of medicine to ensure quality medicament. Thus, there is an urgent need to evaluate such parameters which can be adopted by the pharmaceutical industries. There are reports on standardization of some popular Ayurvedic medicines.[1,2,17,18]

In the communication an attempt has been made to standardize the raw materials as well as finish product of *EG* using modern bioanalytical tools. The results for proximate analysis of raw materials for parameters like ash values (total ash, acid insoluble ash) were found in compliance with pharmacopoeial limits. Values of proximate parameters for *EG* are tabulated in Table 2.

Some of the discerning anatomical characters of the raw materials were observed in the finished *EG* [Figure 2]. Preliminary qualitative phytochemical evaluation of *EG* revealed the presence of carbohydrates, alkaloids, flavonoids, essential oils, glycosides and tannins, while resins were not detected.

Among the solvent systems tried, mixture containing toluene: ethyl acetate: formic acid (6:3:0.3, v/v/v) gave the best resolution for piperine (R_f=0.43) from the formulation matrix which enabled its quantification as well as phytochemical fingerprint. [Figure 3] The identity of the band of piperine in *EG* and was confirmed by comparing UV absorption spectra with that of the standard [Figure 4].

Piperine content of *EG* was determined by HPTLC densitometric method. The method was validated in terms of precision, repeatability and accuracy as per ICH guidelines [Table 3]. Response for Piperine was found to be linear in the range of 20-80 µg mL^{-1} with a correlation coefficient (r^2 value) of 0.991 [Figure 4] which resulted as a regression equation, y=11.838×-98.064. This equation was used to determine respective piperine content of *EG* and its marketed samples. The assay results of the same are represented in Tables 4-6.

Developed HPTLC densitometric method was found to be

Table 2: Proximate analysis of *Eladi Gutika*

Parameters	*Eladi Gutika*	Suggested limits
Total Ash	3.20±0.26	Not more than 4%
Acid insoluble ash	0.52±0.02	Not more than 1%
Water soluble ash	1.48±0.03	Not more than 2%

Table 3: Method validation parameters

Parameters	Result
Linearity range (µg mL^{-1})	20-80
Slope (m)	11.838
Intercept (c)	-98.064
Correlation coefficient (R)	0.9991
LOD (µg mL^{-1})	2
LOQ (µg mL^{-1})	6
System Suitability (*n*=5, % CV)	0.09
Instrument Precision (*n*=6, % CV)	0.74
Intraday (precision) (*n*=3, % CV)	0.85
Interday (precision) (*n*=3, % CV)	0.29
Specificity	Specific
Ruggedness	Rugged

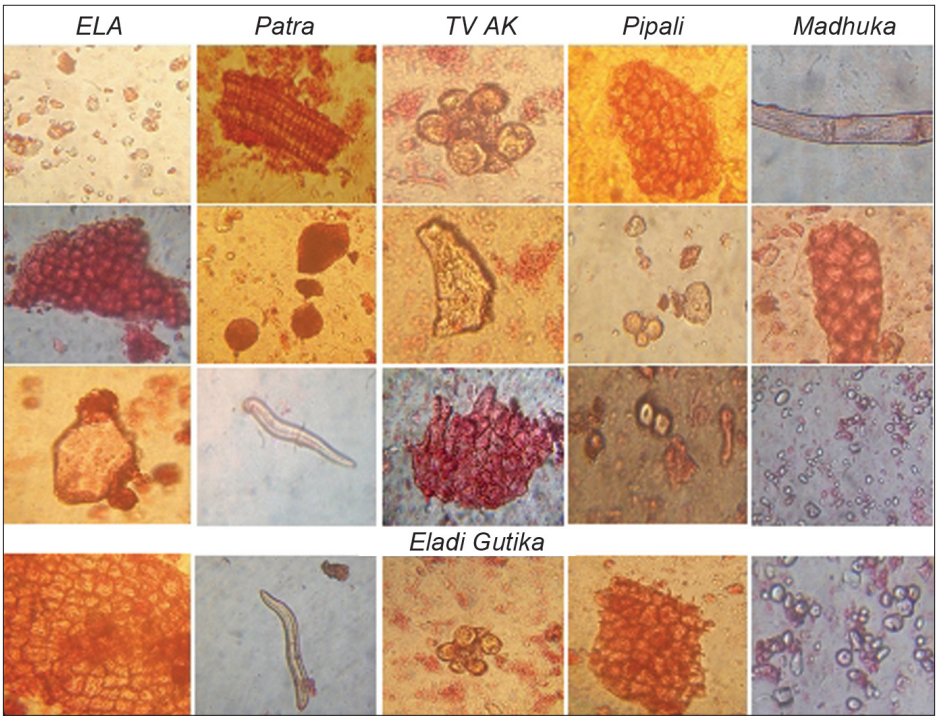

Figure 2: Microscopic characters from *Eladi Gutika* and its ingredients

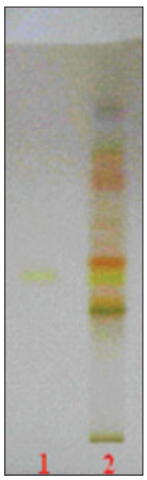

Figure 3: Detection of piperine from *Piper longum* and *Eladi Gutika* at 550 nm using HPTLC, track 1 – Piperine standard, track 2 – *Eladi Gutika*

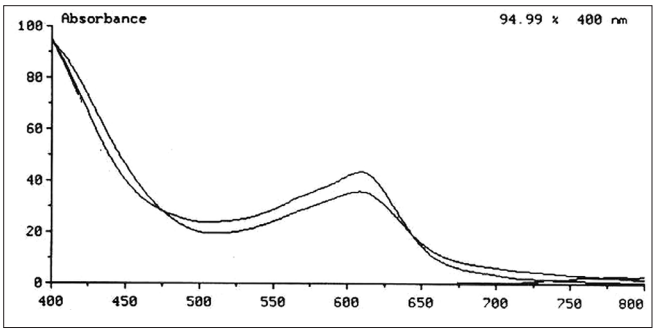

Figure 4: Absorption spectra of piperine with *Piper longum* and *Eladi Gutika*

precise with % RSD < 2 % for intra- and inter-day precision [Table 3]. LOD and LOQ value for Piperine was found to be

Table 4: Amount of piperine in *Eladi Gutika* and *Piper longum*

Sample	Plant part used	Amount of piperine (mg/g), Mean±SD, n=3
Piper longum	Fruit	1.9197 ± 0.0978
Eladi Gutika	–	0.0761 ± 0.0134

Table 5: Comparison of amount of piperine for in-house *Eladi Gutika* and marketed samples

Sample of *Eladi Gutika*	Amount of piperine (mg/g) Mean±SD, *n*=3
In-house	0.0761±0.0134
M-01	0.0747±0.0345
M-02	0.0258±0.0786
M-03	0.0281±0.0876

Table 6: Stability study of in-house *Eladi Gutika*

Stability samples of *Eladi Gutika*	Amount of piperine (mg/g) Mean±SD, *n*=3
0 day	0.0363±0.0978
30 day	0.0437±0.0765
60 day	0.0761±0.0456

Table 7: Results of accuracy/recovery analysis

Excess standard added to the matrix of *Eladi Gutika* (%)	Recovery (%)
0	–
10	99.17±0.5623
20	98.58±0.0235
30	99.74±0.0663
Mean Recovery	99.16±0.0134

2 and 6 μg mL^{-1} respectively [Table 3]. Average recovery at three different levels of piperine for formulation was found to be 99.16 % [Table 7]. Method was found rugged for the parameters like change in analyst, change in mobile phase composition and change in spotting volume etc. Piperine content was maximum in the in-house *EG* when compared with other marketed formulations [Table 5]. Increase in Piperine content was observed for the stability samples stored at different storage periods [Table 6].

There were no significant changes in the body weight, food and water intake of the animals administered with *EG* when compared with the animals of control group and no mortality was recorded. Thus *EG* was found to be safe at the dose of 2g/kg body weight of the animal.

CONCLUSION

Values and the results of such reproducible modern techniques can make the traditional medicines more acceptable in the local and global market. Thus, rationally designed, carefully standardised, synergistic traditional herbal formulations and botanical drug products with robust scientific evidence can also be alternative to modern medicine.

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