

Research Article

Evaluation of *Mimusops elengi* L. flowers using pharmacognostic approach

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ABSTRACT

Background: *Mimusops elengi* L. (Sapotaceae, Bakul) is an integral part of many Ayurvedic formulations. Flowers of *M. elengi* have been traditionally used for their therapeutic activities like antiulcer, wound healing, antioxidant, etc. Flowers are also reported as a brain tonic, expectorant and have been used for the treatment of asthma. **Materials and methods:** The present study was aimed to evaluate the pharmacognostic profile of *M. elengi* flowers as per the standard methods which included evaluation of proximate characters, heavy metals and chromatographic characterization. The study also included evaluation of safety profile of the plant in Albino Wistar rats as per the OECD test guidelines no. 420. **Results:** Various analytical values for *M. elengi* flowers were established. *M. elengi* flowers were found to be a source of terpenoids and phenolics as per the results of chromatographic characterization. *M. elengi* flowers were also found safe up to an oral dose of 2.0 g/kg body weight in rats. **Conclusion:** The pharmacognostic profile for *M. elengi* flowers obtained as a finding of this research work will contribute in their identification and standardization.

Key words: *Mimusops elengi* L. flowers, physicochemical evaluation, phytochemical evaluation, safety evaluation.

INTRODUCTION

Mimusops elengi L. (Sapotaceae), traditionally known as *Bakul* in Ayurveda, is a small to large evergreen tree found all over different parts of India, Burma and Pakistan. The tree is native to the western peninsula. It is also cultivated as an ornamental tree for its aromatic flowers.¹ Almost all the morphological parts of this plant are traditionally used for the management of a wide range of disorders. The bark is used as a cardio tonic, stomachic, anthelmintic, astringent and cures diseases of the gums and teeth. The flowers show cooling and astringent effect to the bowels and are reported to possess immunomodulatory, hepatoprotective, analgesic, antiasthmatic and wound healing activities. Fruits are also astringent to the bowel and good

for teeth. Seeds of the plant are useful to fix loose teeth and used as a cure for troubles in the head. Roots are aphrodisiac, diuretic and good for gonorrhoea.² *M. elengi* is considered as a sacred plant among Hindus and has obtained important place in religious texts as well as in ancient Sanskrit literature.³ Flowers of *M. elengi* (because of their wide spectrum of therapeutic activities) have been used in Ayurveda to treat various ailments.⁴ These therapeutic activities have been attributed to the diverse group of phytochemical constituents. A literature survey revealed that, *M. elengi* flowers possess triterpenoids, alkaloids, flavonoids, volatile oils, etc.²

Pharmacognosy, has gained immense importance in recent years as it is an efficient tool for the authentication and identification of plant raw materials and therefore evaluation of pharmacognostic parameters. It is an indispensable step when dealing with herbal drugs.⁵ *M. elengi* is regarded as one of the best medicinal plants since each and every part of it is used in various ways to cure a variety of human ailments. On account of this fact, sci-

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entific data is being established on the parts of this plant to ensure reliable use. The pharmacognostic and phytochemical screening data on *M. elengi* stem bark has been reported.⁶ Recently, estimation of triterpene acids using HPTLC from *M. elengi* stem bark has been published.⁷ Identification of phytoconstituents from *M. elengi* flowers by GC-MS has been reported.⁸

A thorough literature survey revealed that the scientific data on pharmacognostic profile of *M. elengi* flowers remains less explored. Establishment of pharmacognostic profile of *M. elengi* flowers will assist in their standardization in terms of quality, purity and sample identification.⁹ Therefore, the main objective of this study was to standardize *M. elengi* flowers for their macroscopic, physicochemical, phytochemical, chromatographic, mass spectroscopic and safety profile. Based on the reported therapeutic activities of lupeol (anti-inflammatory, anticancer, antimicrobial, hepatoprotective, cardioprotective,¹⁰ wound healing,¹¹ etc), β -sitosterol (anticancer, estrogenic, hypolipidemic,¹² wound healing,¹³ etc), ursolic acid (anticancer, anti-inflammatory, antioxidant, hepatoprotective,¹⁴ etc), gallic acid (antioxidant, anti-allergic, anti-inflammatory, anticancer, wound healing, etc¹⁵), quercetin (antiallergic, anticancer and immunomodulatory,¹⁶ antibacterial, wound healing,¹⁷ etc) and kaempferol (antidiabetic and anticancer,¹⁶ wound healing,¹⁸ etc); chromatographic characterization of *M. elengi* flowers was carried out in terms of these triterpenoids (ursolic acid, β -sitosterol and lupeol), phenolic compound (gallic acid) and flavonoids (quercetin and kaempferol) content.

The essential oils from the flowers were extracted as per the method reported and its characterization was carried out using GC-MS technique.¹⁹ Further, in order to evaluate safety of *M. elengi* flowers, an acute oral toxicity study (as per OECD test guideline no. 420) was conducted in albino Wistar rats.

MATERIALS AND METHODS

Plant sample

Fresh flowers of *M. elengi* were collected from D.G. Ruparel College, Matunga, Mumbai. The taxonomic identification of a representative sample was confirmed by Agharkar Research Institute, Pune (Authentication no. 14-004). The plant sample was shade dried for four days followed by drying in an oven preset at 45°C for four days. The sample was powdered in a mixer grinder, sieved through 85 mesh (BSS) and stored in an air tight container.

Chemicals and reagents

Ursolic acid, β -sitosterol, lupeol, gallic acid, quercetin and kaempferol (98 % purity each) were procured from Sigma Aldrich Chemical Company, (Steinheim, Germany). Chemicals of analytical grade were purchased from Merck Specialties Private Limited, Mumbai.

Macroscopic and physicochemical evaluation

Macroscopic characters of *M. elengi* flowers such as petals, calyx, androecium, and gynoecium were studied. The physicochemical parameters of the *M. elengi* flowers such as foreign organic matter, loss on drying, ash content (total, acid insoluble and water soluble) and extractive values were determined using standard pharmacopoeial methods.^{20,21}

Phytochemical and heavy metal evaluation

Qualitative phytochemical screening of some major secondary metabolites (flavonoids, essential oils, tannins, glycosides, alkaloids and resins) in *M. elengi* flowers was carried out by performing preliminary phytochemical tests as per the methods reported.²¹ Further, powder of dried *M. elengi* flowers was subjected to quantitative phytochemical evaluation by successive Soxhlet extraction with various organic solvents in order to analyze the percent extract of major class of compounds present in the plant raw material as per the method reported.²² *M. elengi* flowers were also evaluated for the presence of four heavy metals namely lead, arsenic, cadmium and mercury (as recommended by AYUSH) using inductively coupled plasma-optical emission spectroscopy (ICP-OES) technique.²³

Chromatographic evaluation

Extraction of phytochemical constituents from *M. elengi* flowers

The powdered sample (2.0 g) was extracted with methanol (10.0 mL), vortex mixed for a minute and sonicated for 20 min followed by filtration through Whatman filter paper no. 1. The filtrate was subjected to HPTLC analysis for the development of a phytochemical fingerprint. In order to extract the triterpenoids ursolic acid (Figure 1A), β -sitosterol (Figure 1B) and lupeol (Figure 1C) from the complex matrix of *M. elengi* flowers, the powdered sample (2.0 g) was extracted with 10.0 mL petroleum ether,^{24,25} vortex mixed for a minute and then sonicated for 20 min followed by filtration through Whatman filter paper no. 1. Filtrate was evaporated under vacuum using rotary evaporator to dryness at 40°C, reconstituted in equal volume of methanol and subjected to HPTLC analysis for the separation of these triterpenoids. In order to extract the phenolic compound gallic acid (Figure 1D) and flavonoids quercetin and kaempferol (Figure 1E and 1F

respectively) the powdered sample (2.0 g) was extracted with methanol (10.0 mL), vortex mixed for a minute and sonicated for 20 min followed by filtration through Whatman filter paper no. 1. The filtrate was subjected to HPTLC analysis for the separation of gallic acid, quercetin and kaempferol.

Preparation of standard stock solutions

A Denver analytical balance (Goettingen, Germany) was used to weigh the standards. Standard stock solution of lupeol, β -sitosterol, ursolic acid, gallic acid, quercetin and kaempferol (1000.0 $\mu\text{g}/\text{mL}$ each) was prepared in methanol. Serial dilution of the stock solution in methanol was carried out in order to prepare calibrant/quality control samples.

Optimized chromatographic conditions for phytochemical fingerprint and quantitation of markers

The HPTLC system used consisted of CAMAG TLC Scanner 4 supported by winCATS software version 1.4.7 equipped with CAMAG Linomat 5 sample spotter and CAMAG Reprstar 3 system for photo-documentation. Chromatographic separation of the phytochemical constituents was achieved on TLC plates (E. Merck) pre-coated with silica gel 60 F₂₅₄ (0.2 mm thickness) on aluminium sheet support. To develop an HPTLC fingerprint of *M. elengi* flowers, the sample (10.0 μL) was applied to the plate as a band of 8.0 mm wide and at a distance of 15.0 mm from the edges. Each plate was developed up to a distance of 85.0 mm in CAMAG twin trough glass chamber pre-saturated with the mobile phase chloroform: toluene (5:5, v/v) for 15 min. After development, the plate was dried in a current of air at room temperature. The plate was derivatized using 10% methanolic sulphuric acid and dried in oven preset at 110 °C for 10 min. For densitometric scanning, the source of radiation was a mercury lamp (366 nm). All measurements were performed at $22 \pm 1^\circ\text{C}$. Plate was photo-documented at 254 nm (before derivatization), 366 nm (before and after derivatization) and 550 nm (before and after derivatization).

For simultaneous separation of ursolic acid, β -sitosterol and lupeol from *M. elengi* flowers, 10.0 μL of sample along with the standards - ursolic acid (10.0 $\mu\text{g}/\text{mL}$), β -sitosterol (50.0 $\mu\text{g}/\text{mL}$) and lupeol (10.0 $\mu\text{g}/\text{mL}$) of 10.0 μL each were spotted on TLC plate as bands of 8.0 mm wide and at a distance of 15.0 mm from the edges under similar instrumental conditions. The plate was developed up to a distance of 85.0 mm in CAMAG twin trough glass chamber pre-saturated with mobile phase toluene: methanol

(8:1, v/v) for 15 min and derivatized using 10% methanolic sulphuric acid. The post derivatization procedure was kept similar to that used for the development of the fingerprint. Each plate was photo-documented at 366 nm and scanned.

To separate gallic acid from *M. elengi* flowers, the sample (10.0 μL) and gallic acid standard (100.0 $\mu\text{g}/\text{mL}$, 10.0 μL) were spotted on TLC plate as bands of 8.0 mm wide and at a distance of 15.0 mm from the edges under similar instrumental conditions. The plate was developed up to a distance of 85.0 mm in CAMAG twin trough glass chamber pre-saturated with mobile phase toluene: ethyl acetate: formic acid (2:7:1, v/v/v) for 15 min. The plate was scanned and photo documented at 254 nm.

For simultaneous separation of quercetin and kaempferol from *M. elengi* flowers, the sample (10.0 μL) along with standards - quercetin and kaempferol (50.0 $\mu\text{g}/\text{mL}$, 10.0 μL each) were spotted on to the TLC plate as bands of 8.0 mm wide and at a distance of 15.0 mm from the edges under similar instrumental conditions. The plate was developed up to a distance of 85.0 mm in CAMAG twin trough glass chamber pre-saturated with mobile phase toluene: acetone: formic acid (5:2:0.5, v/v/v) for 15 min. The plate was scanned and photo documented at 254 nm.

Mass spectroscopic evaluation

Extraction of volatile oils from *M. elengi* flowers

In order to establish the quality of *M. elengi* flowers (fresh and dried), the existence of volatile constituents was determined using GC-MS analysis.¹⁹ The sample (3.0 g) was extracted with petroleum ether (10.0 mL) and diethyl ether (10.0 mL) separately, sonicated for 1 h and allowed to stand overnight. Then the mixture was filtered and dried under nitrogen. The residue was reconstituted with 300.0 μL of respective solvents and centrifuged. The supernatant was subjected to GC-MS analysis.

Optimized GC-MS parameters

GC-MS with a TR-5 column of 30 m x 0.25 mm x 0.25 mm film thickness was used for evaluation of essential oils in *M. elengi* flowers. The injector temperature was 290 °C in a split mode 1:100. The temperature program was applied from 60-180 °C with a ramp rate of 3 °C/min and from 180-290 °C with a ramp rate of 10 °C/min. The carrier gas was helium with a flow rate of 1 mL/min. The temperature of the ion source and the interface were 220 °C and 275 °C respectively.¹⁹

Safety evaluation

Safety study of *M. elengi* flowers aqueous slurry was conducted in albino Wistar rats (female, 200-225 g, n=6/

group) as per OECD test guidelines no. 420, fixed dose procedure.²⁶ The study was approved from the Institutional Animal Ethics Committee (CPCSEA/315, approval no.: DG-130624-02). The rats were fasted overnight for 10-14 h and orally administered with aqueous slurry of *M. elengi* flowers (2.0 g/kg). The animals were observed individually during the first 30 min for all reflexes, periodically during the first 48 h with special attention given during the first 4 h (short-term toxicity) and daily thereafter for a total of 14 days (long-term toxicity) for alteration from general behavior and clinical symptoms like alteration of skin and fur texture, ptosis, excessive salivation, breathing problems, diarrhea etc. Daily body weight, food and water intake record was also maintained. The results were compared with the control group (orally administered with distilled water).

Statistical analysis

Microsoft Excel-2007 was used to determine mean, standard deviation (SD), relative standard deviation (RSD) and mean difference during the analysis.

RESULTS AND DISCUSSION

Medicinal plants are the valuable and potent source of unique phytochemicals which are frequently used in the development of drugs against a wide range of ailments. A large fraction of the world population, especially in the developing and underdeveloped countries still depends mainly on medicinal plants mentioned in the traditional systems of medicine.^{4,27} *M. elengi* is a large glabrous evergreen tree characterized by a short, dark and very rough trunk and wide spreading (Figure 2A). Flowers of *M. elengi* are tiny, nearly 2.5 cm across solitary, buds ovoid, acute; pedicels 6.20 mm; calyx 1 cm long; stamens eight, opposite to inner circle of lobes and ovary appressedly silky-pubescent.³ Flowers of *M. elengi* are cream coloured, hairy and scented (Figure 2B), yellowish brown when dried (Figure 2C) and brown in colour when powdered (Figure 2D).

Proximate parameters such as foreign organic matter, loss on drying, ash values (total, acid insoluble and water soluble) and extractive values (ethanol soluble, water soluble and ether soluble) of *M. elengi* flowers were determined (Table 1). The water soluble extractive values were found maximum when compared with ethanol soluble and ether soluble extractives. This suggests the presence of more polar components in the plant (approx. 22%). Flavonoids, alkaloids, and glycosides were found to be present in the methanolic extract of *M. elengi* flowers during qualitative

preliminary phytochemical evaluation. Flavonoids and resins were found to be present in the aqueous extract, whereas tannins and essential oils were found absent in both the aqueous and methanolic extract of *M. elengi* flowers (Table 2). Amongst all the phytochemicals fractions extracted, *M. elengi* flowers were found to be rich in quaternary alkaloids and N-oxides fraction, whereas the fraction of alkaloids were least (Table 1). The flowers were also found to be a source of terpenoids and phenolics, hence were further subjected to chromatographic characterisation using validated HPTLC technique. None of the heavy metals analyzed were detected in the *M. elengi* flowers which may reduce the possible risk of using it as a phytomedicine.

M. elengi flowers have been reported to possess compounds like alkaloids, sterols, triterpenes² phenolics and flavonoids.²⁸ Although TLC and HPTLC based comparative evaluation of *M. elengi* flower extracts using different solvent systems has been reported,²⁹ there is no report available on individual phytochemical constituents belonging to above mentioned group of compounds. As these phytochemicals are reported to possess a wide range of therapeutic potential, in the present research work, chromatographic characterization of *M. elengi* flowers was carried in terms of its phytochemical fingerprint and marker content.

A HPTLC fingerprint was developed from the methanolic extract of *M. elengi* flowers using chloroform: toluene (5:5, v/v) as a mobile phase and it showed 11 components in the form of band at 366 nm after the derivatization of plate ($R_f = 0.09, 0.16, 0.23, 0.27, 0.32, 0.46, 0.56, 0.67, 0.71, 0.82, 0.93$) with blue, yellow, purple, light pink coloured bands (Figure 3D and 3E) which will be helpful in the identification of plant raw material. Before derivatization, it showed one dark band ($R_f = 0.12$) at 254 nm (Figure 3A) while two bands (blue and light pink coloured band at $R_f = 0.30$ and 0.72 respectively) were observed at 366 nm (Figure 3B). After derivatization, it showed only one band (pink coloured band at $R_f = 0.75$) when observed at 550 nm (Figure 3C).

Mobile phase composition of toluene: methanol (8:1, v/v) gave good resolution of three triterpenoids (ursolic acid at $R_f = 0.25$, β -sitosterol at $R_f = 0.46$ and lupeol at $R_f = 0.59$) during HPTLC experiment. From the complex matrix of *M. elengi* flowers, only β -sitosterol and lupeol were found to be separated while ursolic acid was not detected. Though ursolic acid has been reported to be present in *M. elengi* flowers,² findings of this research suggest the need of some more sensitive method to detect

Table 1: Results of physicochemical parameters and content of phytochemical fractions of *M. elengi* flowers

Parameters		Results
Foreign organic matter		0.214 ± 0.018
Loss on drying		10.875 ± 0.246
Ash content	Total	4.854 ± 0.523
	Acid insoluble	0.289 ± 0.181
	Water soluble	2.587 ± 0.034
Extractive value	Ethanol soluble	10.944 ± 0.070
	Water soluble	21.999 ± 0.380
	Ether soluble	2.315 ± 0.044
Phytochemical fractions	Fats and waxes	0.990 ± 0.045
	Terpenoids and phenolics	4.272 ± 0.122
	Alkaloids	0.446 ± 0.097
	Quaternary alkaloids and n-oxides	15.040 ± 0.062
	Fibres	66.464 ± 0.136

Values are (% Mean ± S.D., n=3)

Table 2: Phytochemicals in *M. elengi* flowers detected as per preliminary phytochemical tests

Phytochemical constituents	Tests	Observation	Inference
Flavonoids	Methanolic extract + increasing amount of NaOH	Yellow precipitate	Present
	Methanolic extract + Lead acetate	Yellow precipitate	Present
Tannins	Aqueous extract + 5% FeCl ₃	No deep blue-black colour	Absent
	Aqueous extract + K ₂ Cr ₂ O ₇	No decolouration	Absent
	Aqueous extract + Lead acetate	No white precipitate	Absent
Alkaloids	Methanolic extract + Mayer's reagent	Precipitate forms	Present
Glycosides	Methanolic extract + 1.0 mL H ₂ O + NaOH	Yellow colouration	Present
Essential oils	Methanolic extract + drops of vanillin sulfuric acid	No white crystals	Absent
Resins	Boiled aqueous extract + conc. H ₂ SO ₄	Reddish brown colour	Present

and quantitate this marker. Identification of the two triterpenoids (β -sitosterol and lupeol) in the flowers was confirmed by overlay and colour of band with that of the standard (Figure 4A and 4B) as well as with the chromatograms of standards and sample (Figure 4C and 4D).

For the separation of gallic acid from *M. elengi* flowers, toluene: ethyl acetate: formic acid (2:7:1, v/v/v) was used as a mobile phase which gave well resolved band of gallic acid at $R_f = 0.53$ from other component of plant matrices. The presence of gallic acid in the plant sample was confirmed with the spectra, chromatograms and the R_f of the characteristic band in the plant matrix with that of the standard (Figure 5A, 5C, 5D and 5E).

The mobile phase toluene: acetone: formic acid (5:2:0.5, v/v/v) showed good resolution of two flavonoids (quercetin at $R_f = 0.30$ and kaempferol at $R_f = 0.35$). But these two flavonoids were found absent in the plant matrix during HPTLC experiment (Figure 5B). This may be due to the presence of these flavonoids in their glycosidic form in the plant matrix or presence of some other flavonoids in the plant sample. The HPTLC methods were validated as per ICH guidelines and found rapid, specific, precise, accurate and rugged (Table 3). Regression analysis of calibrant samples for each marker resulted in the form of regression equation which was further used to estimate their content of in *M. elengi* flowers. The content of β -sitosterol was found to be higher in *M. elengi* flowers

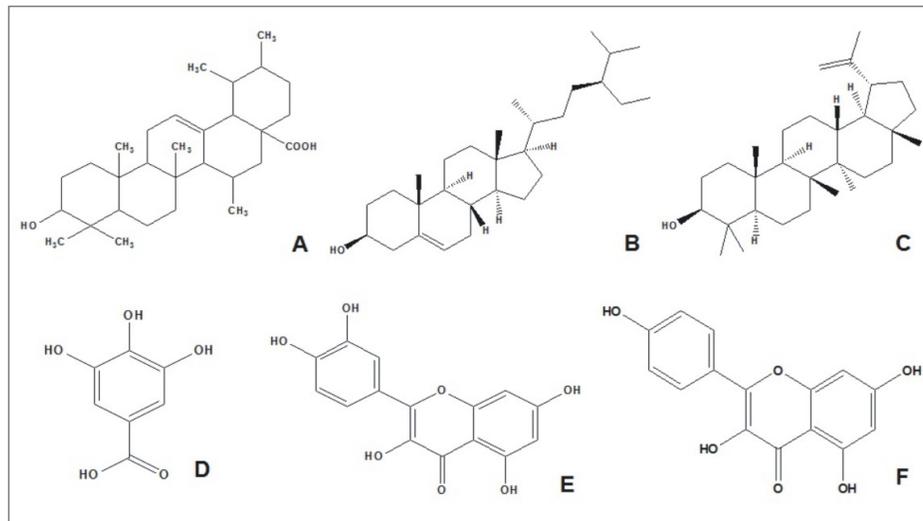


Figure 1: Structure of A) ursolic acid, B) β -sitosterol, C) lupeol, D) gallic acid, E) quercetin and F) kaempferol .

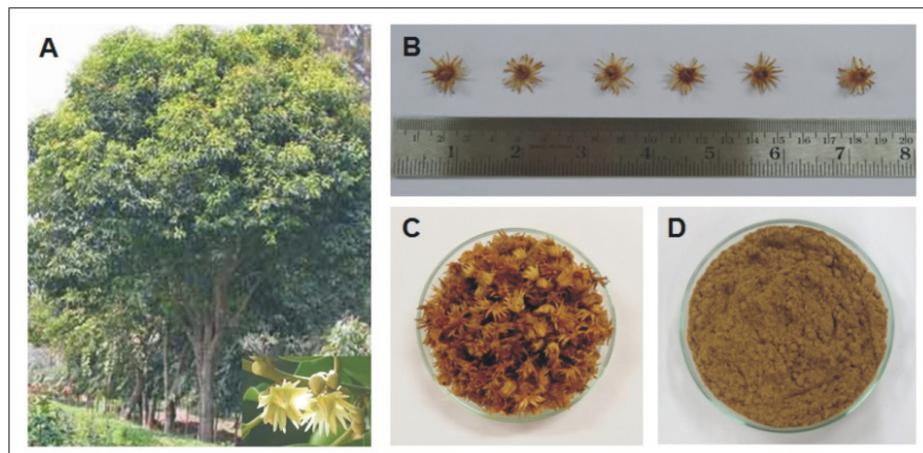


Figure 2: *Mimusops elengi*: A) Habit (inset flowers), B) fresh flowers, C) dried flowers and D) dried and powdered flowers.

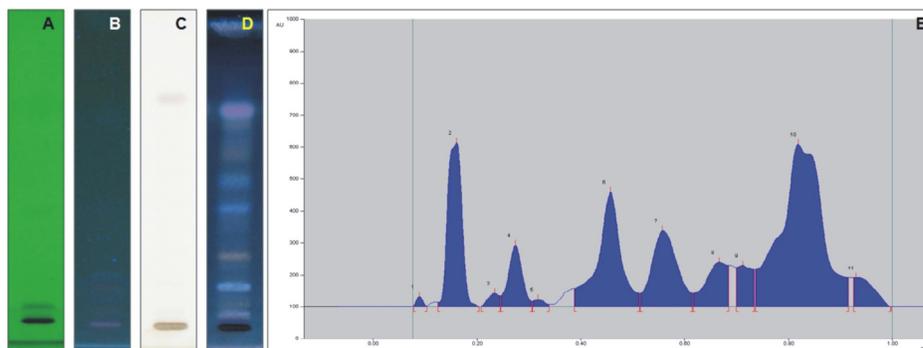


Figure 3: HPTLC plate photo showing the phytochemical fingerprint of *M. elengi* flowers. Plate before derivatization at 254 nm (A), plate before derivatization at 366 nm (B), plate after derivatization at 550 nm (C), plate after derivatization at 366 nm (D) and chromatogram after the densitometric scanning of plate D (E).

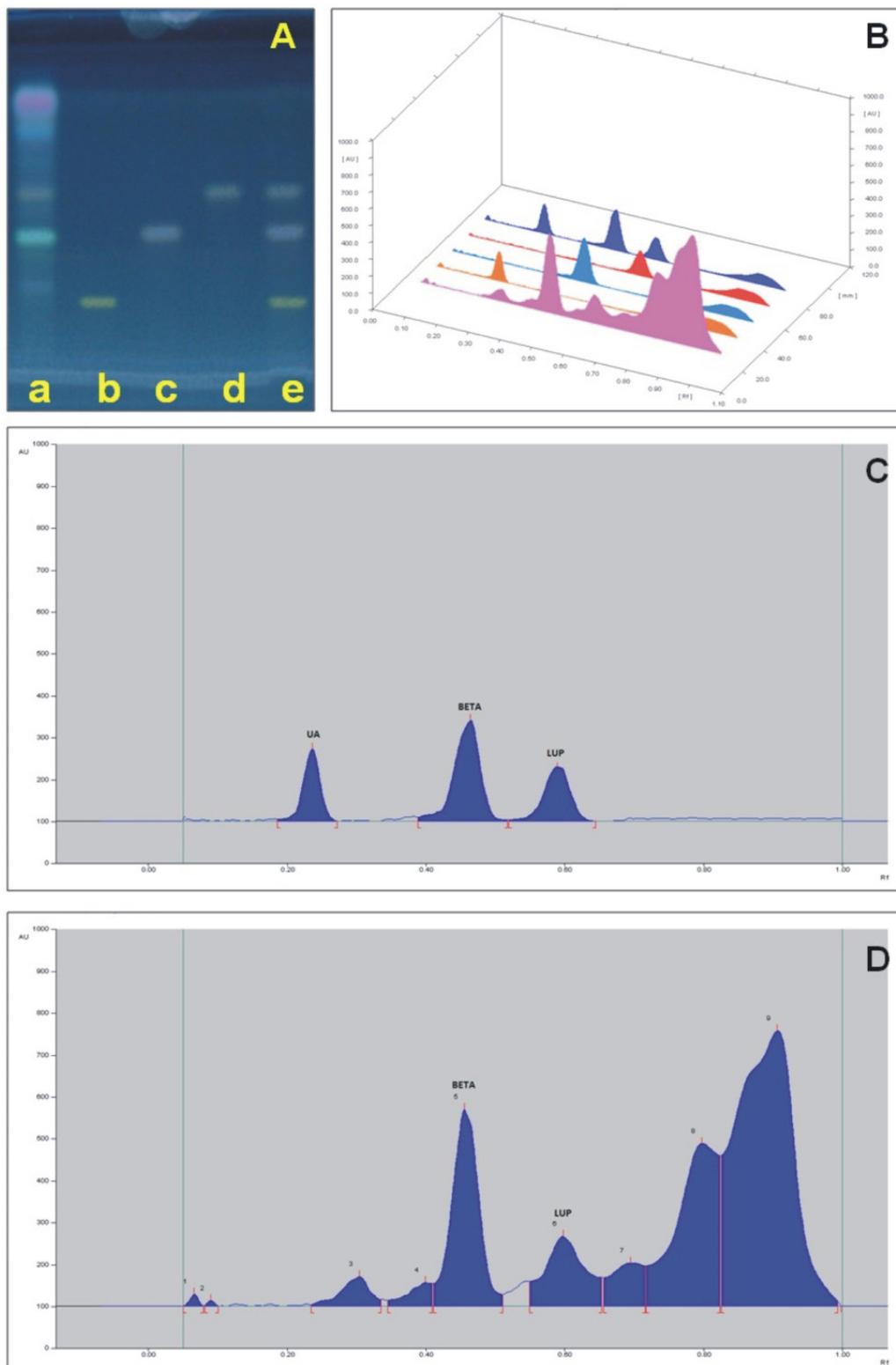


Figure 4: HPTLC plate photo (A) and overlay of the chromatograms (B) showing the presence of two terpenoids - lupeol and β -sitosterol at 366 nm in *M. elengi* flowers. Chromatograms of three terpenoids - ursolic acid, β -sitosterol and lupeol (C) and *M. elengi* flowers showing β -sitosterol and lupeol (D) after the densitometric scanning of plate A at 366 nm. Track details for plate A: a) *M. elengi* flowers collected from D. G. Ruparel College, Matunga b) ursolic acid (10.0 $\mu\text{g}/\text{mL}$), c) β -sitosterol (50.0 $\mu\text{g}/\text{mL}$), d) lupeol (10.0 $\mu\text{g}/\text{mL}$), e) mixture of ursolic acid, β -sitosterol and lupeol (10.0 $\mu\text{g}/\text{mL}$, 50.0 $\mu\text{g}/\text{mL}$ and 10.0 $\mu\text{g}/\text{mL}$ respectively).

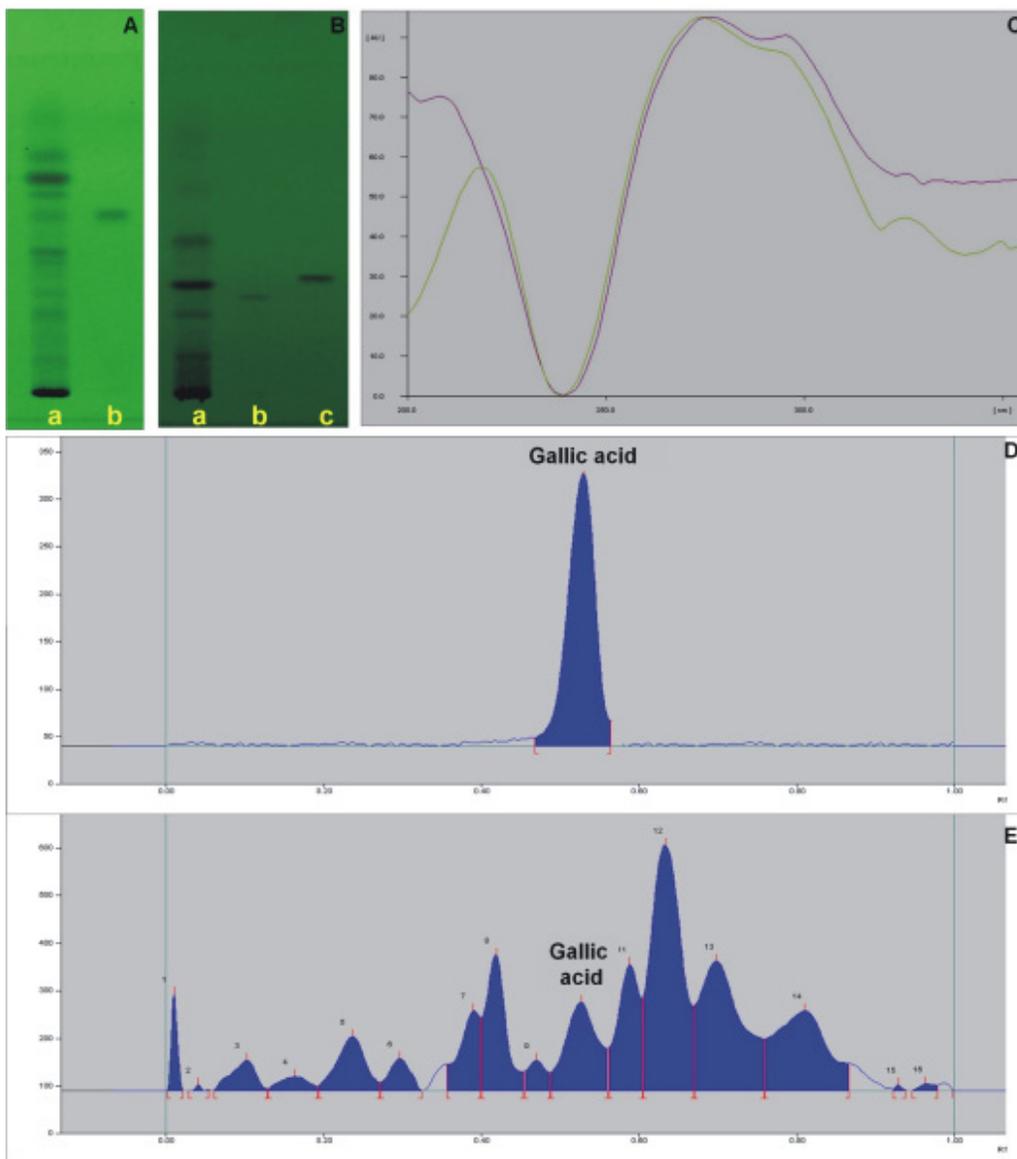


Figure 5: HPTLC plate photo showing the presence of gallic acid in *M. elengi* flowers at 254 nm (A). Plate B represents HPTLC plate photo of *M. elengi* flowers with standards - quercetin and kaempferol. Spectral confirmation of gallic acid in *M. elengi* flowers at 254 nm (C). Chromatograms of gallic acid (D) and *M. elengi* flowers showing gallic acid (E) after the densitometric scanning of plate A at 254 nm. Track details for plate A: a) *M. elengi* flowers collected from D. G. Ruparel College, Matunga, b) gallic acid (100.0 µg/mL). Track details for plate B: a) *M. elengi* flowers collected from D. G. Ruparel College, Matunga, b) quercetin (50.0 µg/mL), c) kaempferol (25.0 µg/mL)

when compared with the content of another triterpenoid, lupeol. The flowers were also found to be a good source of phenolic compound gallic acid (Table 3).

GC-MS analysis enabled the identification of volatile constituents which are the products of secondary metabolism occurring in the plants. These are generally consisting of complex mixtures of terpene hydrocarbons.¹⁹ The number of compounds identified from the petroleum

ether and diethyl ether extract of *M. elengi* flowers (fresh) along with their chemical names are as follows;

In petroleum ether extract: 05 [Trichloroethylene; Cis-9-Hexadecenoic acid; n-Hexadecenoic acid; Squalene; Di-n-octyl phthalate]

In diethyl ether extract: 09 [Benzyl alcohol; 2-phenyl ethanol; 3-phenyl-2-propene-1-ol; 4-hydroxy benzene

Table 3: Results of method validation experiment for simultaneous estimation of triterpenoids (ursolic acid, β-sitosterol and lupeol), phenolic compound (gallic acid) and flavonoids (quercetin and kaempferol)						
Parameters	Results for each phytochemical marker					
	Ursolic acid	β -sitosterol	Lupeol	Gallic acid	Quercetin	Kaempferol
Mobile phase	Toluene: methanol (8:1, v/v)			Toluene: ethyl acetate: formic acid (2:7:1, v/v/v)	toluene: acetone: formic acid (5:2:0.5, v/v/v)	
R_f	0.29	0.50	0.66	0.53	0.30	0.35
LOD ($\mu\text{g/mL}$)	2.5	1.0	2.0	10	2.0	5.0
LOQ ($\mu\text{g/mL}$)	5.0	5.0	5.0	20	5.0	10.0
Linearity ($\mu\text{g/mL}$)	5.0 – 100.0	5.0 – 60.0	5.0 – 75.0	20.0-150.0	10.0-70.0	25.0-250.0
Regression equation	$y = 30.80x + 132.5$	$y = 39.76x + 194.0$	$y = 24.81x + 35.10$	$y = 56.67x - 56.74$	$y = 15.079x - 118.43$	$y = 5.5001x + 254.13$
Coefficient of determination (r^2)	0.999	0.993	0.995	0.992	0.9991	0.9996
Instrumental precision (% RSD), n=7	1.82	1.63	1.57	1.12	0.71	0.61
Repeatability (% RSD), n=5	1.09	0.96	1.28	1.12	0.93	0.75
Intraday Precision (% RSD)	1.73	0.50	0.89	1.37	1.07	1.06
Interday Precision (% RSD)	1.99	0.93	1.15	1.94	1.16	1.12
Recovery (%)	97.23	96.54	98.16	98.12	99.93	98.49
Specificity	Specific					
Ruggedness	Rugged					
Assay (content in mg/g,	Not detected	0.332 \pm 0.002	0.044 \pm 0.001	0.583 \pm 0.007	Not detected	Not detected

Values are Mean \pm S.D., n=3)

methanol; methyl-4-hydroxy benzoate; 2-butyl phenol; n-Hexadecanoic acid; (Z)-9-Octadecanoic acid, methyl paraben]

The number of compounds identified from the petroleum ether and diethyl ether extract of *M. elengi* flowers (dried) along with their chemical names are as follows;

In petroleum ether extract: 09 [(7E)-1-Chloro-7-heptadecene; Eugenol; 3-allyl-2-methoxy phenol; Cis-9-Hexadecenoic acid; n-Hexadecenoic acid; Cis-9,cis-12-Octadecadienoic acid; Tetratetracontane; Cholesta-4,6-diene-3 β -ol; 1,2-Benzenedicarboxylic acid, diisooctyl ester]

In diethyl ether extract: 07 [3-Methoxy-1,2-propanediol; Cis-9-Hexadecenoic acid; n-Hexadecenoic acid; 9-Thiabicyclo(3.3.1)nonane,9,9-dioxide; Octadecanoic acid; Squalene; 1,2-Benzenedicarboxylic acid, diisooctyl ester]

All the compounds have been detected for the first time from the petroleum ether and diethyl ether extract of *M. elengi* flowers (dried) and petroleum ether extract of *M. elengi* flowers (fresh) following the reported extrac-

tion procedure, especially the therapeutically active compound eugenol³⁰ which has not been reported by previous researchers.³¹⁻³³ Safety of *M. elengi* flowers was established in rats using acute toxicity study. Oral administration of *M. elengi* flowers (aqueous slurry) did not cause any mortality as well as no significant change in the body weight, food and water intake was observed when compared with the animals of the control group. The cage side observations also appeared normal.

CONCLUSION

The evaluation of pharmacognostical parameters may ensure the authenticity of *M. elengi* flowers. The validated HPTLC methods for estimation of triterpenoids (ursolic acid, β -sitosterol and lupeol), flavonoids (quercetin and kaempferol) and phenolic compound (gallic acid) can be applied to various herbal drugs for the quantitation of these markers. Findings of the HPTLC analysis of *M. elengi* flowers showed that the plant is a good source of β -sitosterol, lupeol and gallic acid and GC-MS analysis showed the presence of some volatile constituents like

eugenol from *M. elengi* flowers. These compounds are reported to possess potent wound healing properties. Results of the acute oral toxicity study may ensure an adequate safety margin of *M. elengi* flowers for their intended use. The overall findings of this study could be useful for proper identification and quality control of *M. elengi* flowers. Thus, *M. elengi* flowers being a good source of triterpenoids (β -sitosterol and lupeol) and phenolic compound (gallic acid) and eugenol may further be evaluated for their wound healing potential.

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