



Pharmacognostic Evaluation of *Vatankur* - An Ayurvedic Drug

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ABSTRACT

The leaf bud of *Ficus benghalensis* L. (Moraceae) is commonly known as *Vatankur*. It is traditionally used in the treatment of diarrhoea, skin disorders, female reproductive disorders and as a hair vitalizer. In the present study macroscopic, microscopic, proximate, phytochemical and safety profile of *Vatankur* has been evaluated and discussed. Findings of this study can provide reference information on *Vatankur* for its correct identification and quality evaluation.

Keywords: Pharmacognosy, Phytochemical evaluation, Safety, *Vatankur*.

INTRODUCTION

Ficus benghalensis L. (Moraceae) is a large evergreen tree distributed throughout India. It has numerous aerial roots arising from horizontally growing branches and attains a height of 20 m or more with a stout trunk.¹ In Ayurveda, the leaf bud of *F. benghalensis* (*Vatankur*) is used as an anti-diarrhoeal agent and in the management of skin and female reproductive disorders. The drug is also used as a hair vitalizer.² It is one of the important ingredients used in the processing of base oil for any *Taila* (oil based Ayurvedic preparation).³

Pharmacognosy is the scientific study of crude drugs⁴ and pharmacognostic evaluation of medicinal plants involves evaluation of many parameters such as morphological and anatomical characters⁵⁻⁶, proximate and physicochemical parameters⁷, preliminary phytochemical screening⁸, chromatographic profile⁹⁻¹¹, safety and pharmacological evaluation¹²⁻¹³ etc.

Recently, a preliminary report on physicochemical and phytochemical evaluation of *Vatankur* has been published along with some data on microscopic characters¹⁴ but the data on morphological and anatomical description, chromatographic and safety profile of *Vatankur* remains an unexplored issue. Hence, the main objective of the present study was to evaluate various pharmacognostic parameters of *Vatankur*.

MATERIALS AND METHODS

Sample collection and processing

Fresh leaf buds of *F. benghalensis* were collected from Kalyan, authenticated by the Department of Botany, Ramnarain Ruia College, Mumbai and a representative voucher specimen was deposited (HRL/Auth/12/13) at the centre. In order to study the impact of regional variation, samples were also collected from different regions of Maharashtra. Samples were shade dried for a week and kept in an oven preset at 45°C, powdered,

sieved (BSS mesh number-85) and stored in air tight containers.

Quality evaluation of *Vatankur*

Macroscopic and microscopic evaluation

The fresh leaf buds were evaluated for the macroscopic characters such as size, shape, colour, presence or absence of stipule, surface, apex, margin, etc. Transverse sections of the fresh leaf buds were taken on clean glass slides, stained in dilute safranin, mounted in glycerol, examined and photographed under light microscope (100x). The powdered sample was also analyzed for its microscopic characters.

Physicochemical and phytochemical evaluation

The proximate characters of *Vatankur* were determined as per the standard methods.¹⁵⁻¹⁷ Content of some major secondary metabolites in *Vatankur* was determined as per the reported method.¹⁸

Optimized chromatographic conditions for estimation of lupeol from *Vatankur*

- Extraction of phytochemical constituents from *Vatankur* collected from different regions: Powder of *Vatankur* (1.0 g) + Solvent (10.0 mL of methanol) → kept standing overnight → filtered through Whatman filter paper no. 1 → filtrate used for HPTLC analysis.
- Standard: Lupeol (98 % purity, Sigma Aldrich) in methanol (w/v).
- Stationary phase: Merck Silica gel 60 F₂₅₄ HPTLC pre-coated plates of 0.2 mm thickness with aluminium sheet support.
- Mobile phase: Toluene: methanol, 8: 1 (v/v).
- Equipment details: Sample spotter [CAMAG Linomat 5 with syringe (Hamilton)], Visualizer (CAMAG UV Cabinet), Photodocumentation unit (CAMAG



Reprostar 3), Scanner (CAMAG Scanner 4 with winCATS planar chromatography manager software version 1.4.7), Wavelength (366 nm).

- Derivatizing reagent: 10 % methanolic sulphuric acid.
- Method validation: The HPTLC method was validated as per ICH guidelines.¹⁹⁻²⁰

Safety evaluation

- Heavy metal analysis: *Vatankur* was also analyzed for the presence of heavy metals like lead, arsenic, mercury, cadmium using ICP-OES technique.²¹
- Acute oral toxicity study: *In vivo* acute oral toxicity of *Vatankur* was conducted²² and the results were compared with the animals of control group treated with distilled water. [Guidelines: OECD guideline No. 420, Ethical Committee approval no.: CPCSEA-315/PP-120806-01, Animals: Swiss Albino mice, female (20-28 g), n=6/group, Parameters observed: Cage side observations, change in body weight along with food and water intake and mortality]

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

Macroscopic and microscopic characters of *Vatankur*

Macroscopically, *Vatankur* consists of 12-15 young leaves in a whorled arrangement. The entire leaf bud is covered by a convoluted stipule (Figure 1a and 1 b).

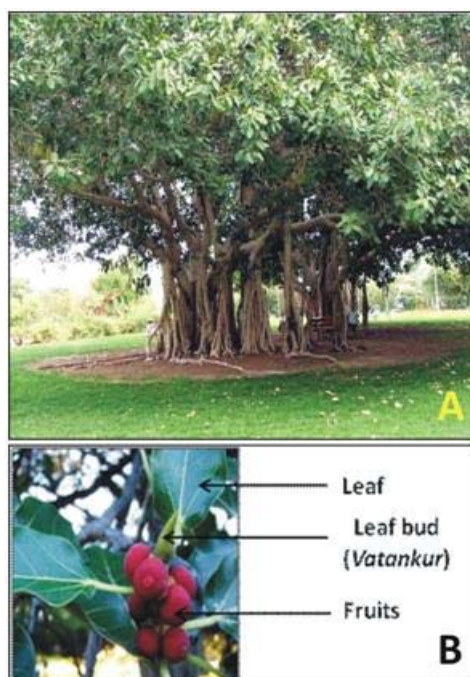


Figure 1: Macroscopic characters of *Ficus benghalensis*. A) Habit, B) Twig showing leaf bud (*Vatankur*), leaves and fruits

The length of the bud ranges from 1.3 to 4.5 cm and width from 0.5 to 1.3 cm. It is conical in shape and light to dark green in colour (Figure 2a and 2b). The young leaves are simple, opposite, petiolate and elliptical with an acute apex. Margins are entire, even and smooth. Surface is hairy with unicostate reticulate venation. The dried leaf buds were pale yellowish to brown in colour. The powder of *Vatankur* appeared light brown in colour with characteristic odour and taste (Figure 2c).

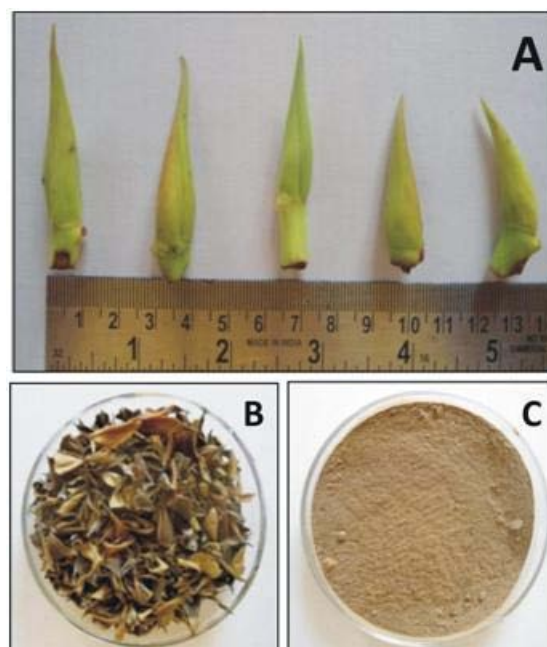


Figure 2: *Vatankur*. A) Fresh, B) Dried, C) Powdered

Microscopic evaluation is one of the cost effective and simplest methods for the correct identification of the drug⁵. The transverse section of *Vatankur* revealed typical anatomical characters of a leaf. It showed a single layer of epidermis on both the surfaces and was covered with cuticle. The mesophyll tissue was clearly differentiated into palisade and spongy cells along with the presence of vascular bundles. Palisade cells were more prominent than spongy. Presence of oil globules within mesophyll and unicellular trichomes towards the upper epidermis were characteristic feature of *Vatankur* (Figure 3a and 3b). Anatomical characters such as unicellular trichomes, spiral vessels, pitted fibres, stone cells, etc were abundant in powdered drug of *Vatankur* (Figure 3c). Results were in compliance with the earlier published report.¹⁴ These morphological and microscopic characters will enable to identify *Vatankur* in crude as well as in powdered form.

Physicochemical and phytochemical parameters of *Vatankur*

Proximate parameters such as loss on drying, foreign matter, ash values (total, acid insoluble and water soluble) and extractive values of solvents with varying polarities (water, methanol, chloroform, ethyl acetate, toluene and n-hexane) for *Vatankur* were determined and results are shown in Table 1. Variation in the major phytochemicals extracted from *Vatankur* was observed

and the results are summarized in Table 2. *Vatankur* was found rich in quaternary alkaloids and n-oxides among the other phytochemicals extracted. *Vatankur* consisted of 81.57 % of fibres which may be from cellular walls, sclerenchyma and transporting tissues. Approximately 90.52% of the phytochemicals from *Vatankur* were extracted using the reported method.

Table 1: Physicochemical parameters of *Vatankur*

Parameters	Results (%Mean \pm SD, n=3)	
Foreign matter	0.25 \pm 0.02	
Total ash	7.48 \pm 0.05	
Acid insoluble ash	0.38 \pm 0.03	
Water soluble ash	4.53 \pm 0.21	
Loss on drying	0.09 \pm 0.01	
Extractive values	Water	10.60 \pm 0.59
	Methanol	7.45 \pm 0.23
	Chloroform	1.32 \pm 0.09
	Ethyl acetate	1.44 \pm 0.08
	Toluene	4.20 \pm 0.80
	n-hexane	1.84 \pm 0.31

Table 2: Data for major phytochemicals extracted from *Vatankur*

Parameters	Results (%Mean \pm SD, n=3)
Fats and waxes	1.24 \pm 0.07
Terpenoids and phenolics	2.15 \pm 0.09
Most alkaloids	0.74 \pm 0.04
Quaternary alkaloids and n-oxides	4.82 \pm 0.88
Fibres	81.57 \pm 1.44

Chromatographic characterization of *Vatankur*

Till date, there is no report on the evaluation of the phytochemicals from *Vatankur*. Leaves of *F. benghalensis*

have been reported to possess rutin, bergapten, friedelin, taraxosterol, β -sitosterol, psoralen, lupeol etc¹. Based on the reported therapeutic activities of lupeol in the management of diarrhoea²³, skin and female reproductive disorders²⁴ and available report on the phytochemical constituents in the leaves of *F. benghalensis*; our recently reported HPTLC method²⁰ was used to separate lupeol (Figure 4a) from other interfering phytochemical constituents of *Vatankur*.

Briefly, the separation of lupeol was achieved from methanolic extract of *Vatankur* on HPTLC plates using toluene: methanol (8:1, v/v) as the mobile phase and 10 % methanolic sulphuric acid as a derivatizing reagent (Figure 4b). Lupeol was detected at $R_f = 0.58$ and its identity in the matrix of *Vatankur* was confirmed by overlay and colour of band with that of the standard. The method was validated as per ICH guidelines and was found to be rapid, specific, precise, sensitive and rugged during the validation experiment (Table 3).

Table 3: Results of HPTLC method validation for the estimation of lupeol

Parameters	Results
Specificity	Specific
LOD (μ g/mL)	2.0
LOQ (μ g/mL)	5.0
Linear working range (μ g/mL)	5.0 - 50.0
Regression equation	$y = 15.935x + 14.412$
Coefficient of differentiation (r^2)	0.992
Instrumental precision (% RSD, n = 7)	1.27
Repeatability (% RSD, n = 5)	0.89
Intra-day precision (% RSD, n=3)	1.11-1.41
Inter-day precision (% RSD, n=3)	1.25-1.39
Average recovery (% Mean \pm SD, n=3)	97.11 \pm 0.941
Ruggedness	Rugged

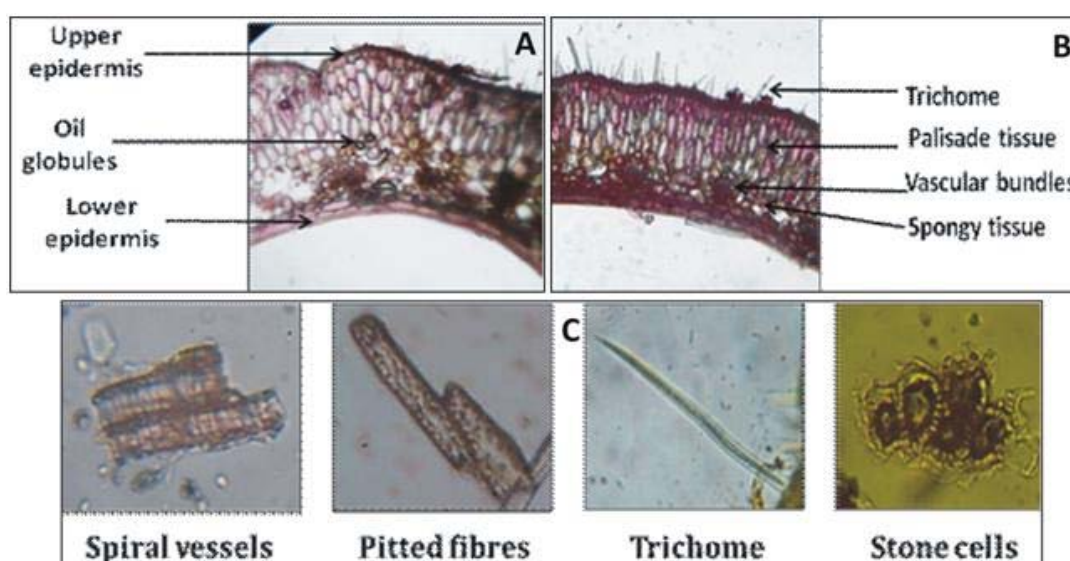


Figure 3: Transverse section of *Vatankur* (fresh) showing A) Upper epidermis, oil globules and lower epidermis B) Trichome, palisade tissue, spongy tissue and vascular bundles C) Powder microscopy of *Vatankur*

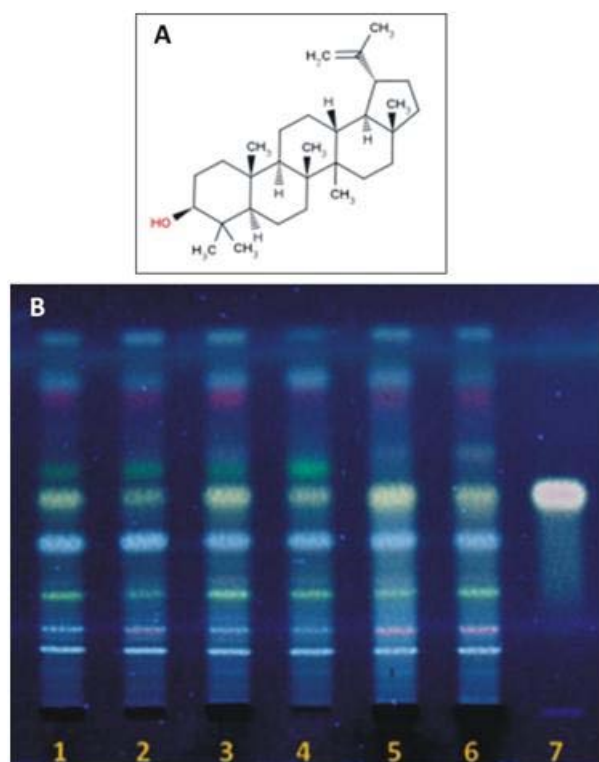


Figure 4: A) Structure of lupeol B) HPTLC plate photo of *Vatankur* collected from different regions of Maharashtra with lupeol at 366 nm

Using the regression equation, the exact content of lupeol in *Vatankur* was determined. Samples of *Vatankur* collected from different provinces of Maharashtra were used to evaluate the effect of regional variation on the content of lupeol. Samples of *Vatankur* collected from different geographical location were not identical in terms of their lupeol content. The content of lupeol was found maximum in the sample collected from Murud while sample from Dadar had minimum content (Figure 4b and Table 4). The HPTLC fingerprint of the *Vatankur* along with lupeol within the specified limits can be used to verify its quality and purity. This method can be applied to various polyherbal formulations of any matrices containing lupeol.

Table 4: Content of lupeol in the samples of *Vatankur* collected from different regions of Maharashtra

Regions	Content of lupeol in mg/g (%Mean \pm SD, n=3)
Ambarnath	0.25 \pm 0.011
Dadar	0.12 \pm 0.005
Kalyan	0.30 \pm 0.007
Matunga	0.16 \pm 0.009
Murud	0.34 \pm 0.012
Thane	0.22 \pm 0.006

Safety profile of *Vatankur*

Vatankur was analyzed for the presence of four heavy metals which were found to be below the detection limits of the instrument. In acute toxicity studies, oral administration of *Vatankur* did not cause any mortality as well as no significant change in the body weight, food and

water intake when compared to the animals of control group. The cage side observations (condition of the fur/skin/teeth, pupil diameter, colour and consistency of faeces, gait, etc) appeared normal. Since toxicity in humans cannot always be entirely extrapolated from animal studies, clinical evaluation should be performed to precisely define the safe dosage. Results of heavy metal analysis and acute oral toxicity study revealed that *Vatankur* could be considered with a wide margin of safety for oral use. Thus, the results of pharmacognostical evaluation could be useful as quality control method for the determination of identity, purity, authenticity and adulteration (if any) for *Vatankur*.

CONCLUSION

The pharmacognostical parameters reported here can be considered as distinctive enough to identify and decide the authenticity of *Vatankur* in herbal industry. Lupeol, a pharmacologically active marker in *Vatankur* has been evaluated through validated HPTLC method. Thus, findings of this study could be useful for the compilation of a monograph in suitable pharmacopoeia for its proper identification and quality control.

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