

Phyto-Pharmacognostical Studies and HPTLC Fingerprinting Profile of *Uvaria narum* (Dunal) Wall. Ex Wight

Kalpna Sabanna Patil* and Raju Ratan Wadekar

ABSTRACT

Objective: To study the pharmacognostic characters and phytochemical investigation of medicinally important crude drug, *Uvaria narum* (Dunal) Wall. Ex Wight (Annonaceae). **Methods:** Organoleptic characters, microscopic evaluation, leaf constants, quantitative physico-chemical evaluations (Ash value, extractive value, moisture content), preliminary phytochemical screening, quantitative estimation of total phenolic and flavonoid content in alcoholic and aqueous extract of *Uvaria narum* and HPTLC fingerprinting profile were studied on leaves of *Uvaria narum* by employing standard methods of analysis. **Results:** Leaf is dorsiventral, having distinct upper and lower surface. Stomata are of paracytic type and present only on lower epidermis. Upper epidermal cells are polygonal with cuticle and contain simple starch grains. A single layer of palisade cells is present below upper epidermis. Leaf does not bear any trichomes. Mid rib region shows collenchymatous cells on both surfaces. Xylem bundles are arranged in an arc. The space between collenchymas and vascular bundle is occupied by parenchymatous cells, some of which are filled with calcium oxalate crystals of prism as well as of rosette type (30-40 μm in diameter). HPTLC fingerprint study of alcoholic and aqueous extracts confirmed the presence of quercetin as biomarker polyphenolic compound. **Conclusion:** These findings will be useful in establishing pharmacognostic and phytochemical standards for correct identification, as well as assessment of purity, quality of this plant, which definitely gaining the relevance in plant drug research and establishment of plant monograph.

Key words: Histochemical Evaluation, HPTLC Fingerprinting, Morpho-Anatomical, Physico-chemical, *Uvaria narum*.

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INTRODUCTION

Pharmacognostic study deals with the selection, authentication, collection and quality evaluation of crude drugs and herbal materials based on macroscopic and microscopic characters.¹ During the past few decades, advances in chemical and biological techniques of analysis have been greatly transformed research in the field of Pharmacognosy.² On the other hand, physicochemical and phytochemical investigation of plant material and their phytoconstituents plays important role in the field of drug discovery of phytopharmaceuticals.³ Current global scenario suggests that, in many developing countries, a large group of population relies on traditional practitioners and medicinal plants to fulfill their primary health care needs. Furthermore, many people in developed countries believe in alternative and complementary therapies, including medicinal herbs.⁴ However, a key impediment, for the worldwide acceptance of the alternative medicines, is lack of documentation and stringent quality control. There is a need for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely

important to make an effort towards systematic standardization of the plant material to be used as medicine.⁵

Uvaria narum (Dunal) Wall; belonging to family Annonaceae, is commonly found in southern India mainly in Kerala. It is known as Kariballi in Kanada, Nilavalli in Sanskrit and Pulliccan in Tamil. In Ayurveda, it is widely used in the treatment of ailments such as eczema, jaundice and swelling. The leaves are useful in vitiated conditions of *vata* and *pitta*, inflammation and fever.⁶ A study was conducted for the evaluation of antibacterial and antioxidant properties of *Uvaria narum* by using various extracts. The root extracts showed remarkable degree of antioxidant activity, and antibacterial properties against *Staphylococcus aureus*, *Escherichia coli*, and *Lactobacillus fermentum* spp.⁷

Extensive literature search revealed lack of systematic Pharmacognostic and phytochemical standardization of leaves of *Uvaria narum*. Thus, present study was undertaken to carry out detailed phyto-pharma-

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cognostical standardization of this plant for its correct authentication and identification.

MATERIALS AND METHODS

Plant material and extraction

The plant material was collected from the vicinity of Pune district. Botanical identification of the taxa was carried out using available literature survey whereas; authentication of medicinal plant was done by Sr. taxonomist from Botanical Survey of India, Pune. The voucher specimen number is BSI/WC/Tech/2008/354-RRW/UN-III. The herbarium was submitted at the department of Pharmacognosy, K.L.E University's College of Pharmacy, Belgavi. After authentication, the leaves of *Uvaria narum* were dried at room temperature until they were free from moisture and subjected to morpho-anatomical and physicochemical studies. Subsequently, the powder material of *Uvaria narum* extracted successively by using solvent of increasing polarity viz; petroleum ether, chloroform and alcohol. Whereas, Chloroform water IP 1996 was used for preparation of aqueous extract.

Chemicals and instruments

Phloroglycinol, Hydrochloric acid, 5% Iodine, Sudan red and all other chemicals were of analytical grade. Instruments: Microscope (Make-Zeiss Company with Axis Vision Ac Rel 4.5 Software), Microtome (Make: Thermo Electron Corporation and Model Shandon Finesse) and CAMAG (Muttentz, Switzerland) HPTLC including a Linomat V sample applicator, a Camag twin trough plate development chamber, Camag TLC scanner 3 and WIN CATS-4 integration software.

Organoleptic evaluation

The leaves of *Uvaria narum* were subjected to morphological studies composed of organoleptic characteristics viz, color, odour, taste, shape, texture were examined as per standard WHO guidelines.⁸

Microscopical evaluation

For microscopical studies, the required plant sample was cut and removed from healthy plant and washed with water. After proper washing the killing and fixing of the specimen was carried out using solution of (90 ml 70% ethanol + 5 ml of glacial acetic acid + 5 ml of formaldehyde) for one week. Further, dehydration of the tissues was done with the help of different grades of tertiary butyl alcohol. Thereafter, the process of infiltration was followed by filling the cells with increasing order of paraffin. Furthermore, thin transverse sections was taken using microtome (Make: Thermo Electron Corporation and Model Shandon Finesse) and histochemical tests were carried out using staining reagents such as phloroglycinol + hydrochloric acid (1:1) for (lignified cells), 5% Iodine for (starch grains), Sudan red for (stone cells). Photomicrographs of the microscopical sections were captured with the help of microscope (Make- Zeiss Company with Axis Vision Ac Rel 4.5 Software).⁹

Quantitative Leaf constants and physical evaluation

Microscopic leaf constants such as, palisade ratio, vein islet number, vein termination number and stomatal index were evaluated by using Camera Lucida apparatus. Furthermore, the shade dried leaves were subjected to size reduction to get fine powder (# 40 size mesh) and subsequently evaluated for physical constants viz; ash value, extractive value and moisture content as per the literature.¹⁰

Qualitative and quantitative phytochemical analysis

The preliminary phytochemical analysis on alcoholic and aqueous extracts of leaves of *Uvaria narum* has been carried out by using qualitative chemical test.¹¹ Whereas, the alcoholic and aqueous extracts were subjected for quantitative estimation of total phenolic and total flavonoids content.

Determination of total phenolic content

The alcohol and aqueous leaf extracts of *Uvaria narum*, (10 μ L) were mixed with 0.2 mL Folin-Ciocalteu reagent, 2 mL of water, and 1 mL of 15% Sodium carbonate, and the mixture was measured at 765 nm after 2 h at room temperature. The mean of the three readings was used and the total phenolic content is expressed in milligram of gallic acid equivalents/1 g extract. The coefficient of determination is $r^2 = 0.9958$.¹²

Determination of total flavonoid content

The flavones and flavonols in alcohol and aqueous leaf extracts of *Uvaria narum*, were expressed as quercetin equivalent. Quercetin was used to make the calibration curve (0.04, 0.02, 0.0025 and 0.00125 mg/mL) in 80% ethanol (v/v). The standard solutions or extracts (0.5 mL) were mixed with 1.5 ml 95% ethanol (v/v), 0.1 mL 10% aluminium chloride (w/v), 0.1 ml of 1 mol/L Sodium chloride was substituted by same volume of distilled water in blank. After incubation at room temperature for 30 min, the absorbance of reaction mixture was measured at 415 nm. The mean of the three readings was used and the total flavonoid content is expressed in milligram of quercetin equivalents/1 g extract. The coefficient of determination is $r^2 = 0.9961$.¹³

HPTLC fingerprinting

HPTLC study was carried out on aqueous and alcoholic extract of leaves of *Uvaria narum*.¹⁴

Development of solvent system

A number of solvent system/mobile phase were tried individually as well as in combination for separation and identification of quercetin from the respective extracts but the satisfactory resolution was obtained in Ethyl acetate: Formic acid: glacial acetic acid: Water (99:12:11:27 v/v/v).

Sample application and development of chromatogram

The aqueous and alcoholic extracts of *Uvaria narum*, was dissolved in respective HPTLC grade ethanol and water which were used for sample application on precoated silica gel GF 254 aluminium sheets (Made-Merck). The samples (5 μ L) were spotted in the form of bands of width 6 mm with a 100 μ L sample using a Hamilton syringe on silica gel which was precoated on aluminium plate GF-254 plates (20 cm X 10 cm) with the help of Lineman 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software. Plates were developed using mobile phase. Linear ascending development was carried out in (20 cm X 10 cm) twin through glass equilibrated with mobile phase.

Derivatization and Detection of spots

The developed plates were dried with the help of hot air oven. Visualization of spot was made after derivatization with 5% Ferric chloride solution. Further, the plates were dried at 100°C in hot air oven for 3 min. and subsequently kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images under UV light at 366 nm, respectively. The R_f values and finger print data were recorded by WIN CATS software.

RESULTS

Organoleptic evaluation

South-Indian *Uvaria* is found in Western ghats from Maharashtra southwards up to an altitude of 1,200 m. *Uvaria narum* is a large woody stellately, pubescent straggling shrub with dark bluish green leaves. Leaves are oblong-lanceolate, pointed or long-pointed hairless on both sides, stalks short, less than 6 mm. Crushed leaves smell like cinnamon. (as shown in Figure 1).

Microscopical and Histochemical evaluation

Transverse section of leaf showed a dorsiventral (having distinct upper and lower surface) pattern. Stomata are of paracytic type and present only on lower epidermis. Upper epidermal cells are polygonal with cuticle and contain simple starch grains (3-5 μ). No stomata are present on upper epidermis. A single layer of palisade cells is present below upper epidermis. Next to palisade layer is a single row of cell containing rosette calcium oxalate crystals and remainder of the mesophyll consists of spongy parenchyma. Xylem bundles are arranged in an arc. The space between collenchymatous and vascular bundle is occupied by parenchymatous cells, some of which are filled with calcium oxalate crystals of prism as well as of rosette type (30-40 μ) in diameter as shown in Figure 2a, 2b and 2c.

Powdered drug analysis

The powder was dark green in colour, with aromatic odour, and taste. After shaking the powder with water in test tube, no persistent foam was formed indicating absence of saponins. Powdered drug under ultra-violet and ordinary light when treated with different reagent emitted various colour radiations which help in identifying the drug in powder form. Behaviour of powder with different chemical reagents is summarised in Table 1.

Histochemical analysis of powdered crude drug revealed presence of calcium oxalate crystals of varying shape such as rosette and prism, starch grains, and paracytic type of stomata as shown in Figure 3.

Quantitative microscopy and physicochemical evaluation

The stomatal index and palisade ratio were found to be 25.2-26.6 and 1-6 respectively. Whereas vein islet and vein termination number were ascertained as 28-30 and 60-65 respectively. The ash content of drug also showed presence of in-organic compounds such as: calcium, magnesium and sulphate while absence of sodium, potassium and phosphate types of inorganic compounds. The physicochemical parameters such as ash value, extractive value and moisture content of the drug summarized in Table 2.

Qualitative and quantitative phytochemical investigation

Successive soxhlet extractive values colour and consistency of extracts of leaves of *Uvaria narum* was found to be: Pet ether (3.09% w/w, greenish, sticky mass); Chloroform (4.22% w/w, yellowish- green, sticky mass); alcohol (5.10% w/w, greenish, sticky mass) and water (5.20% w/w, greenish-black, semi-solid), respectively.

Preliminary phytochemical analysis of alcoholic extract revealed presence of alkaloids, tannins, flavonoids whereas, aqueous extract showed presence of polyphenolic compounds, flavonoids, tannins and glycosides. The quantitative estimation of total phenolic content in alcohol and aqueous extract was found to be 208 ± 2.8 and 212 ± 4.5 mg/g of extract and the quantitative estimation of total flavonoid content in alco-

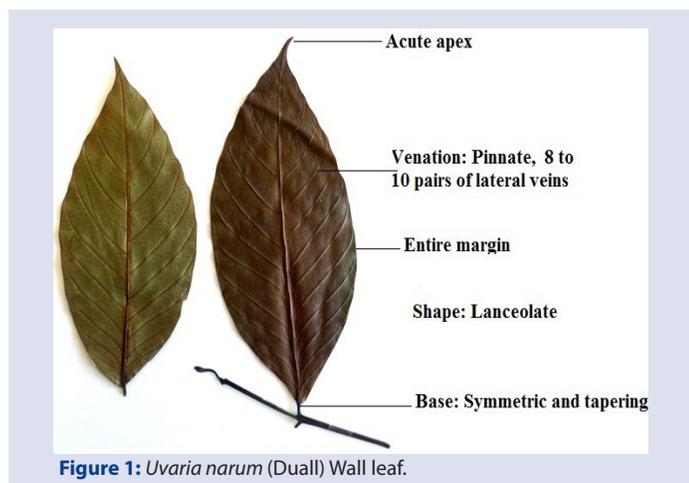


Figure 1: *Uvaria narum* (Dunal) Wall leaf.

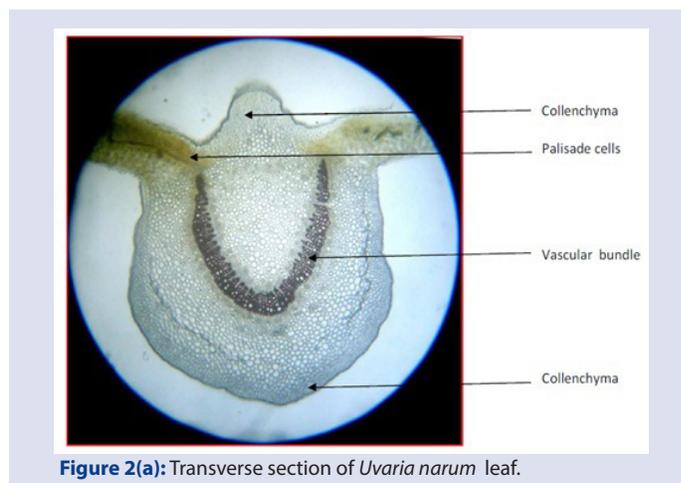


Figure 2(a): Transverse section of *Uvaria narum* leaf.

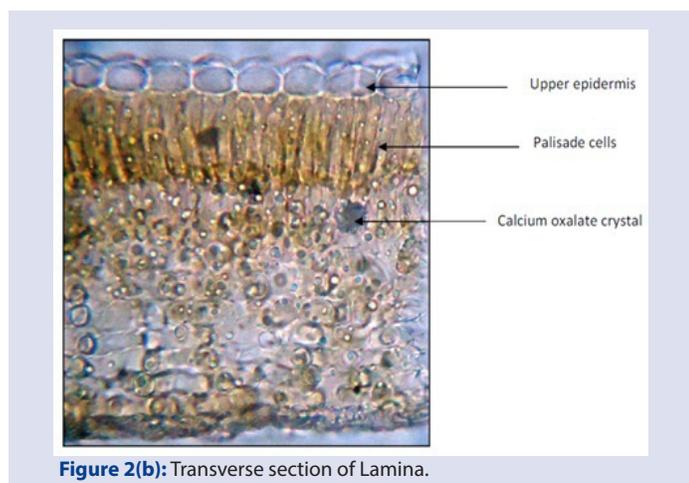


Figure 2(b): Transverse section of Lamina.

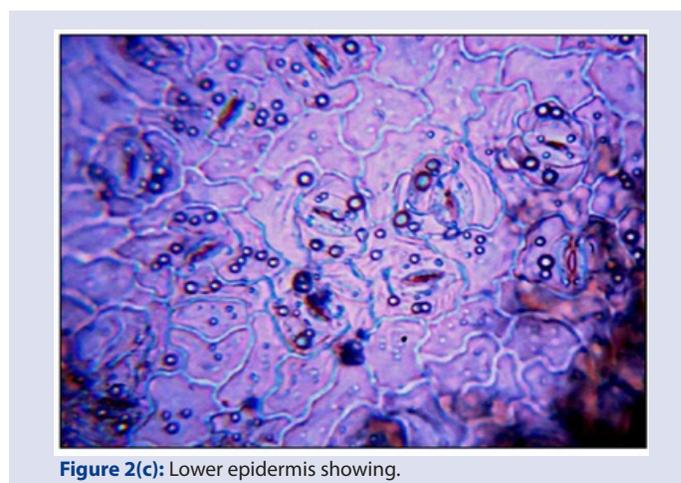


Figure 2(c): Lower epidermis showing.

hol and aqueous extract was found to be 42 ± 3.5 and 60 ± 6.2 mg/g of extract.

HPTLC analysis

The HPTLC chromatogram at 214 nm showed presence of quercetin and exhibited blackish (visible) band in the Rf range of 0.47 to 0.52. According to the literature of flavonoids the Rf range are found to be 0.4 to 0.6. Therefore, the Chromatogram fingerprint suggests the presence of quercetin in aqueous and alcoholic extract of *Uvaria narum* as shown in Figure 4a, 4b, 4c and 4d).

DISCUSSION

The objective of the present study was established and developed the rigorous phyto-pharmacognostic standards for correct identification and authentication of medicinally important *Uvaria narum* plant. Some of

the important diagnostic features of leaf are its aromatic odour, presence of calcium oxalate crystals of prism as well as of rosette type, paracytic type of stomata, starch grains and absence of trichomes were also observed. Determination of Leaf constants plays a crucial role in the study of quantitative microscopy and they can be used to differentiate closely related other *Uvaria* species. The establishment of physical constants viz; moisture content, ash value, acid-insoluble value and extrac-

Table 1: Fluorescence analysis of powdered leaves of *Uvaria narum*

Treatment	Observation under		
	Ordinary light	UV light	
		254 nm	366 nm
Powder as such	Dark green	Green	Dark green
Powder + nitrocellulose	Dark green	Light green	Dark green
Powder + 1N NaOH in methanol	Light yellow	Yellow	Dark Yellow
Powder+ 1N NaOH in methanol + nitrocellulose in amyl acetate	Florescent	Light Florescent	Dark Florescent
Powder + 1N HCl	Light brown	Light brown	Brown
Powder + 1N HCl + nitrocellulose in amyl acetate	Brown	Light brown	Dark brown
Powder + 1N NaOH in water	Yellow	Light green	Light green
Powder + 1N NaOH in water, dried and mounted in nitrocellulose in amyl acetate	Yellowish brown	Light yellowish brown	Dark yellowish brown
Powder + HNO ₃ (1:1)	Brown	Brown	Brown
Powder + H ₂ SO ₄ (1:1)	Light black	Black	Black

Table 2: Physicochemical evaluation of powder leaves of *Uvaria narum* Wall

Parameters	% w/w (Mean a± SEM)
Total ash	4.39 ± 0.31
Acid insoluble ash	0.42 ± 0.02
Water soluble ash	1.01 ± 0.02
Alcohol soluble extractive	20.21 ± 0.22
Water soluble extractive value	22.19 ± 0.21
Moisture content	8.1 ± 0.88

^aMean value of three readings.

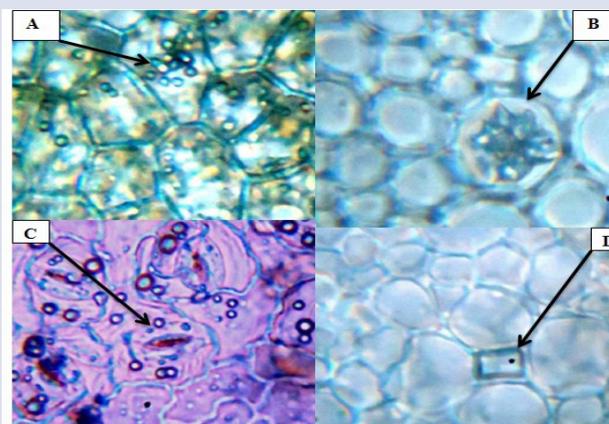


Figure 3: Microscopic powder characteristics of *Uvaria narum* Leaf.

- A:** Starch grains,
- B:** Rosette type calcium oxalate crystal,
- C:** Paracytic type of stomata and
- D:** Prism type calcium oxalate crystal.

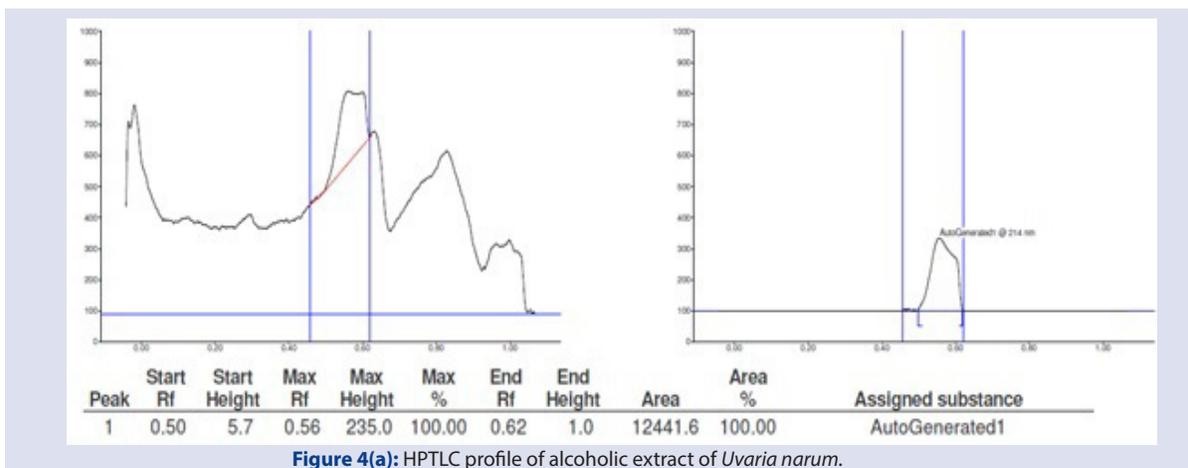


Figure 4(a): HPTLC profile of alcoholic extract of *Uvaria narum*.

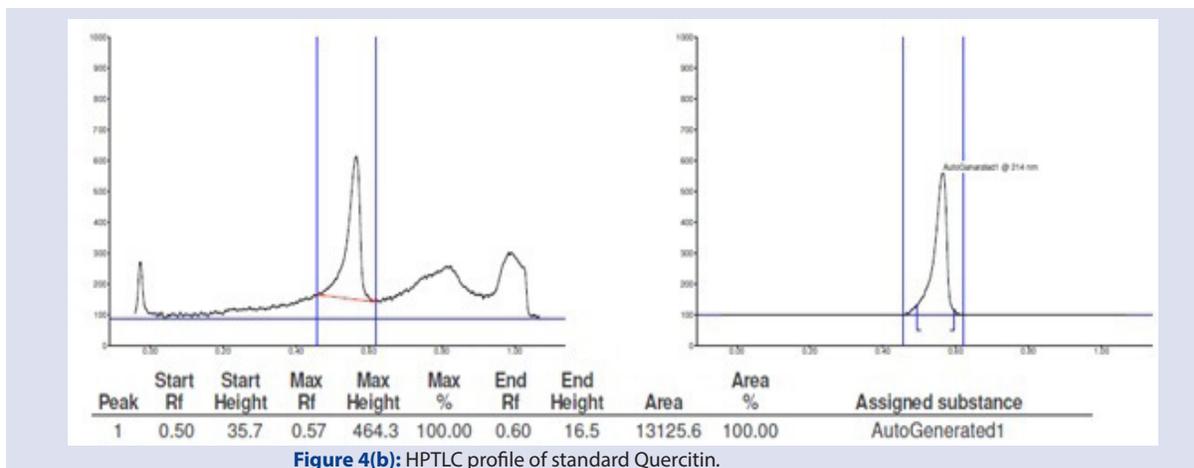


Figure 4(b): HPTLC profile of standard Quercetin.

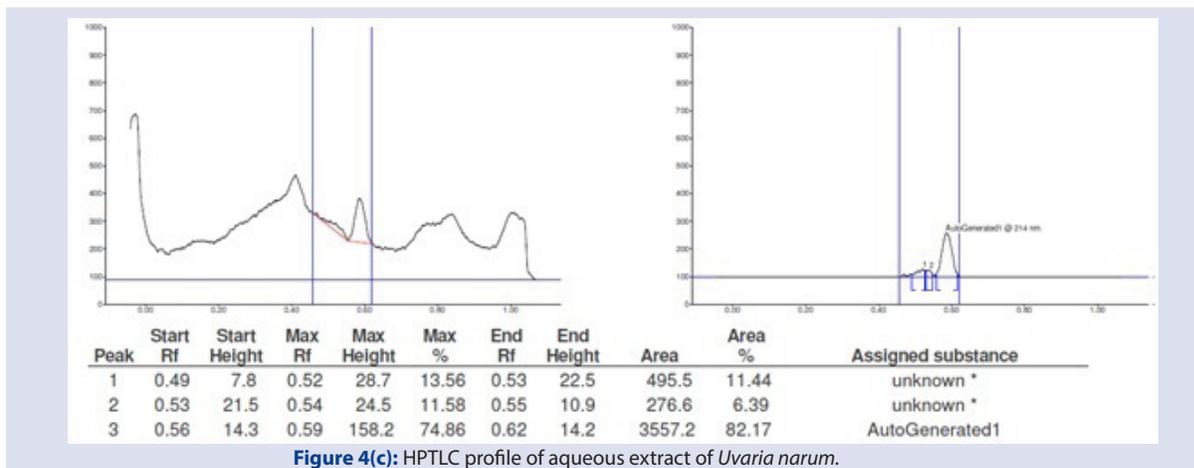


Figure 4(c): HPTLC profile of aqueous extract of *Uvaria narum*.

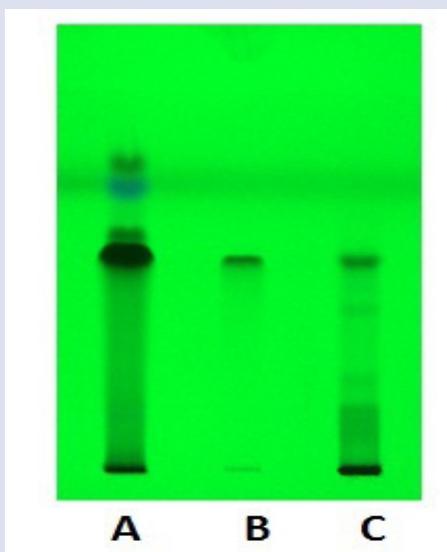


Figure 4(d): HPTLC Chromatogram of:
A: Alcoholic extract of *Uvaria narum* (UNAL);
B: Quercetin as standard
C: Aqueous extract of *Uvaria narum* (UNAQ) scanned at 366 nm.

tive value has equal importance in the evaluation and identification of inorganic impurities present in the crude drugs.¹⁵ Plant serves as a biochemical laboratory for the biosynthesis of major secondary metabolites such as alkaloids, tannins, polyphenolic compounds that exhibits significant physiological effects. Presence of important plant secondary metabolites such as tannin, phenolic substances, steroids, glycosides in *Uvaria narum*, could make the plant useful for treating different ailments of living organism.¹⁶ Thus qualitative and quantitative phytochemical screening along with HPTLC study may be helpful in the detection of bioactive principles.¹⁷ The leaf has shown remarkable amount of polyphenolic present in alcoholic and aqueous extracts. An important observation from Phytochemistry point of view is presence of quercetin in the leaf quantified by using HPTLC.

CONCLUSION

The present investigation yielded a set of qualitative and quantitative phyto-pharmacognostic parameters along with HPTLC fingerprinting profile that can serve as diagnostic tools for establishment of quality standards, authentication and identification of the medicinally important plant *Uvaria narum* (Dunal) Wall. Ex Wight and could be useful for the compilation of a suitable monograph of this plant.

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CONFLICT OF INTEREST

Authors declared no conflict of interest.

ABBREVIATION USED

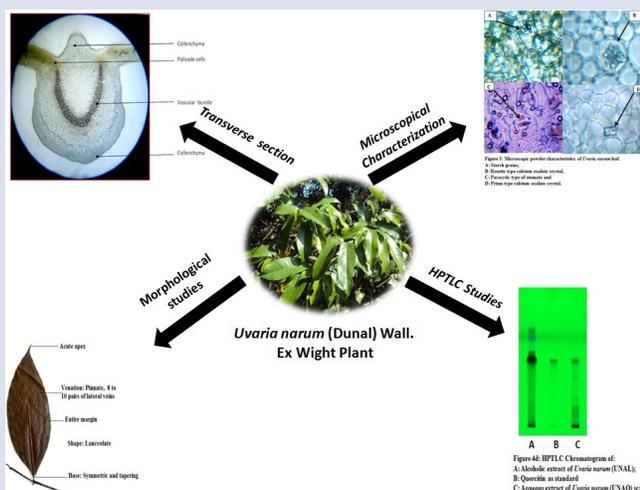
HPTLC: High Performance Thin Layer Chromatography; **TLC:** Thin Layer Chromatography; **Rf:** Retention factor; **UNAL:** *Uvaria narum* alcoholic extract; **UNAQ:** *Uvaria narum* aqueous extract.

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GRAPHICAL ABSTRACT



SUMMARY

- The morpho-anatomical studies on leaves of *Uvaria narum* (Dunal) Wall revealed the presence of the important diagnostic features viz; presence of calcium oxalate crystals of prism as well as of rosette type, paracytic type of stomata, starch grains and absence of trichomes which is the important distinguish characters, for correct authentication and identification of genuine drug.
- Presence of important plant secondary metabolites such as tannin, phenolic substances, steroids, glycosides in *Uvaria narum*, could make the plant useful for treating different ailments of living organism because therapeutic efficiency of any plant is usually trace by their chemical compounds. The leaf has shown remarkable amount of polyphenolic present in alcoholic and aqueous extracts.
- An important observation from Phytochemistry point of view is presence of quercetin in the leaf quantified by using HPTLC. The pharmacognostic constants of this plant and diagnostic microscopic features reported in this work could be useful for the compilation of a suitable monograph and proper identification as well as distinguishing between closely related species

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Kalpana Patil: Is working as Professor at department of Pharmacognosy, K.L.E. University's College of Pharmacy, Belgavi, Karnataka, India. Dr. Kalpana Patil has vast experience in standardization of crude drugs, extraction/Isolation/Characterization of various primary and Secondary plant metabolites and their pharmacological screening for various biological activities and standardization of polyherbal formulations. She has projects in collaboration with national and international institutions.

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