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Pharmacognostical identification of male inflorescence of *Borassus flabellifer* L. (*Panampoo*) sold as *Gajapippali* by macro-microscopy, HPTLC and DNA barcoding

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The female inflorescence of *Scindapsus officinalis* (Roxb.) Schott commonly known as *Gajapippali* is an important Ayurveda drug of Araceae family. A market survey revealed that the inflorescence of *Borassus flabellifer* L., a completely unrelated plant species, is traded as *Gajapippali* owing to its morphological similarities. The botanical identity of medicinal plants is of utmost importance for research as a raw drug's medicinal efficacy and therapeutic properties vary with botanical source. This study aims to validate the key identifying features of the unofficial source of *Gajapippali* with the help of pharmacognostical techniques, including DNA Barcoding. Pharmacognostical fingerprinting of the male inflorescence of *B. flabellifer* was carried out by macro-microscopy, physicochemical analysis, HPTLC, and DNA-based molecular methods to identify the botanical precisely. Morphology was carried out using macroscopy, and the anatomical parameters were studied using microscopy. In addition, phytochemical studies were conducted with the help of HPTLC fingerprinting. The nucleotide sequencing using rbcL marker was carried out for species identification, and a barcode was generated. The morphology of the male inflorescence by macroscopy, tissue arrangements at the cellular level by microscopy of transverse section and powder, purity determination by physico-chemical methods and phytoconstituents fingerprinting using HPTLC showed key identifying features of the inflorescence. The conserved DNA sequence showed the homology of the species, which was submitted to NCBI and converted to a barcode for identification of the botanical. This study will be helpful in the identification and determination of *B. flabellifer*, the market source of Gajapippali, and help in the purity check and quality control of this herbal raw material used in place of the official source.

Keywords: *Borassus*, DNA barcoding, *Gajapippali*, HPTLC, Microscopy, *Panampoo*

IPC code; Int. cl. (2021.01)−A61K 36/00, A61K 36/889

Introduction

There is a growing interest in Ayurveda, Siddha, and Unani (ASU) herbs around the globe. Adulteration and substitution of rare ASU herbs is a serious problem in the herbal pharmacy and pharmaceutical industries and has significantly impacted commercial natural product research and development. Deforestation and extinction of numerous species and inaccurate identification of rare, endangered, and original herbal plants have resulted in adulteration and substituting raw medications[1,2].

Scindapsus officinalis (Roxb.) Schott belongs to the family Araceae[3], which is distributed in the regions of the Eastern Himalayas. It is an epiphytic and perennial climber with adventitious aerial roots that clings to trees and rocks[4]. According to *Bhavaprakasa*, an important Ayurvedic treatise, *Gajapippali*, the female inflorescence of *S. officinalis*, has long been a drug of controversial origin[5]. Some of the medicinal properties of *S. officinalis* are anthelmintic, anti-dysenteric, antiasthmatic, anti-inflammatory, analgesic, antibacterial, carminative, diaphoretic, and hypoglycemic, It is recognized to heal "*atisara*" (diarrhoea), "*svasa*" (dyspnea), "*kanthmaya*" (throat disorders), and "*krimi*" (parasitic infestation) in the Ayurvedic system of medicine[6]. It is of great concern that the drug is often adulterated knowingly or unknowingly due to mistaken identity. Dry or powdered forms of medicinal drugs are difficult to detect, contributing to increased adulteration practices. Therefore, it is imperative to establish adequate guidelines for standardizing herbal

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medications. One common substitute sold in the market is the male inflorescence of *Borassus flabellifer* L., a palm tree belonging to the Arecaceae family. The drug is derived from a dioecious tree having male and female inflorescence on distinct plants. It is known as *Panampoo* in Siddha and is used for treating fevers, hypochondriac disorders, skin diseases, strangury and tooth diseases[7], and is the source of *Tala* in Ayurveda[8]. Common medicinal properties of *B. flabellifer* include analgesic, antipyretic effects, anti-inflammatory activity and immunosuppressant properties[9]. The spadix ash from the palmyra tree is used to reduce the enlarged spleen and liver, as well as the heartburn[10]. Shirisha *et al.*[11] and Gummadi *et al.*[12] has reported analgesic activity in the ethanolic extract of male inflorescence. Paschapur *et al.*[13] have reported the anti-inflammatory activity of male flower ethanolic extract; the ethanolic extract at doses of 150 and 300 mg/kg showed anti-inflammatory response in the mice.

The Consortium for the Barcode of Life (CBOL) plant working group has indeed recommended a standardized plant DNA barcode for land plants, which involves two key genetic markers: rbcL and matK. rbcL (ribulose-1,5-bisphosphate carboxylase/ oxygenase large subunit) gene encodes a subunit of the enzyme ribulose bisphosphate carboxylase/ oxygenase, which plays a key role in the photosynthetic process. It is widely distributed among plants and has been considered a core marker for plant barcoding due to its high recovery rates and relatively well-established sequence databases. It provides good resolution at higher taxonomic levels. rbcL is considered a reliable marker and a good candidate for broad universal barcode due to its unambiguous alignment, higher primer universality and high sequence quality[14,15].

Though different therapeutically, because of their similar morphology, common occurrence and cheap sourcing the male inflorescence of *B. flabellifer* is sold in markets as *Gajapippali*. As a result, it is important to ensure that plant products are properly identified so that consumers reaffirm safety and efficacy.

As a comprehensive macro-microscopy of *B. flabellifer* male inflorescence is not available, hence the present study was conducted to standardize morphological, macro-microscopic, physicochemical, HPTLC and DNA Barcode characters to generate a monographic format for this botanical. This study focuses on the exhaustive differentiation of *B. flabellifer* sold as *Gajapippali* in raw drug markets instead of the authentic *S. officinalis*.

Materials and Methods

A market survey was carried out to procure raw drug samples sold in the name of *Gajapippali* from both the northern and southern states of India. The authentic male inflorescence of *B. flabellifer* was collected from Amoor Kuppam in the Villupuram district, Tamil Nadu (Lat 11.852643° Long 79.32896°). The voucher specimen (I/FL23) was deposited in the museum of the Department of Pharmacognosy, Siddha Central Research Institute (CCRS), Arumbakkam Chennai, for future reference.

Macro-microscopy

The samples procured were examined thoroughly, and the macroscopical characters were observed and recorded using the Nikon (Thailand) Digital Camera D5600. The market and authentic male inflorescences were submerged in water for 24 h, and then free-hand sections were taken following standard procedures. For the microscopic study, both hand sections and microtome section were prepared using Leica RM2125 RTS Rotary microtome. Micro-morphological characters were examined under a Zeiss Axiolab 5 trinocular microscope fitted with an Axiocam 208 colour camera[16].

Sample preparation

Physicochemical parameters were estimated according to the standard pharmacopoeial procedures[17]. One gram of each powdered plant sample was sonicated with 10 mL of methanol for 15 min, filtered, and transferred to a sample vial for HPTLC.

TLC/HPTLC

Exactly 20 µL extract of each male inflorescence of *B. flabillifer* collected from field (Bf), New Delhi (BfND) and Tirunelveli (BfT) were applied on a silica-coated TLC plate (60 F254) using Camag (Switzerland) ATS4 applicator and developed in a Twin Trough chamber (CAMAG) (20× 10 cm), previously saturated with the mobile phase toluene: ethyl acetate: methanol =5:4:1, v/v). The plate was developed till 85 mm from the bottom of the plate. After development, the plate was photographed using a Camag TLC Visualizer at UV 254 nm and UV 366 nm. Then, the plate was scanned using Camag Scanner 4 at 254 nm (D2 lamp, absorption mode) and 366 nm (Hg lamp, fluorescence mode), and the fingerprint profiles of the extract were determined.

Subsequently, the plate was dipped in vanillin sulphuric acid solution in alcohol, followed by heating at 105°C till the development of the coloured spots. The plate was then photographed in white light using a Camag TLC Visualizer2[18].

Extraction of DNA

DNA was extracted from the male inflorescence of *B. flabillifer* collected from Villupuram and the market sample from Delhi using the CTAB method, with a few modifications. The isolated DNA samples were diluted by combining T10E1 with pure genomic DNA before PCR amplification. The amount and purity of the DNA were assessed using NanoDrop (Nanodrop One, ThermoScientific, USA); the isolated DNA was stored at -20°C[19].

PCR Amplification

Based on primer sequences already reported in the literature, rbcL whole primers were used for this study (Forward: 5'ATGTCACCACAAACAGAAAC3' and Reverse: 5'TCGCATGTACCTGCAGTAGC3'). The primer was bought from Bioserve Biotechnologies (India) Pvt Ltd (Telangana). A 25 µL volume was utilized for the PCR reaction, which included 1.5 µL of Taq DNA polymerase (Takara), 10X PCR buffer, 25 mM Mg2+, and 1 µL of genomic DNA as a template. Amplification using thermo-fisher PCR was done in 35 cycles, followed by 94°C for 30 seconds, followed by annealing at 54°C for 45 seconds, and then by extension at 72°C for 45 seconds. After 35 cycles, the profile was linked on hold at 4°C. Then, to check the presence and absence of the band, electrophoresis of the amplified product was made to run on a 2% agarose gel+0.3 µL Ethidium bromide. The band size of amplified products was determined using a 100 bp ladder[20].

The amplified DNA product was then sequenced using NGS in Applied Biosystem Pvt. Ltd.The basic local alignment search tool (BLAST) was used to compare the sequences similarities[19]. The sequences obtained were then submitted to NCBI for future reference.

Results

The male inflorescence of *B. flabellifer* were collected from January to July 2022. Authentic collections were made by field studies from Vilipuram district (BfA), Tamil Nadu. The marketed samples of *B. flabellifer* were collected by a thorough survey. The samples obtained from Tirunelveli (BfT) and New Delhi (BfND) were selected for the pharmacognostical studies along with the field collected sample.

Macroscopy

The macroscopical observation showed different unrelated taxa sold under the name of *Gajapippali*. We could observe pieces of the dried inflorescence of *B. flabellifer* and the inflorescence of *Piper retrofractum* Vahl and *Piper mullesua* Buch.-Ham. ex D.Don (Fig. 1). *P. retrofractum* showed spikes measuring 3.8 to 8.5 cm long and 2.5 to 4.5 mm in diameter with stalked and nearly peltate bracts, while *P. mullesua* showed obovoid drupe, measuring about 2.5 cm in diameter and partly immersed in rachis. As *B. flabellifer* is the official substitute of the original *Gajapippali*, i.e. *S. officinalis*, further studies were focused on *B. flabellifer*. The habitat and macroscopic details of authentic male inflorescences of *B. flabellifer* are depicted in Fig. 2 and key features were compared to the market samples. The cylindrical cut pieces of market sample of spadix male inflorescences varied in sizes from 2 cm to 15 cm in length, 1.5 to 2.5 cm thickness and 1.5 to 2.5 cm in width; dark coffee brown externally and light brown internally; external and internal cut surface rough with numerous shiny scale bracts; fracture short in well-dried condition and fibrous in slightly wet condition; central axil bearing several spiral rows of the bracts were auricular and persistent; flowers staminate, sub-sessile, imbricate, embedded in rachilla pits and concavities concealed by bracts; perianth consisted narrowly cuneate three sepals and shorter spathulate

Fig. 1 — Marketed samples of *Gajapippali* a–c) *B. flabellifer*; d) *P. retrofractum*; and e) *P. mulleusa*.

three petals; stamens 6 arranged in two whorls of three each, filaments free, connate with the corolla into a stalk at the base into a ring; anther are large, sub-sessile, linear, basifixed and longitudinally dehiscent; characteristic smell and an astringent taste. In the market samples, only broken pieces of inflorescence were observed without peduncle. It was noted that there were no flowers on the rachis as they had withered away on drying (Fig. 2).

Microscopy

Inflorescence

The diagrammatic TS of the axis is circular in outline with scaly bracts occupying half of the peripheral region and the central portion of the main axis having parenchymatous ground tissue with fibro-vascular bundles, the rachilla pits periphery area hold matured flowers and young flower buds are embedded towards the inner region, each male flower consists of a chamber encircled by outer and inner whorls of tepals towards its periphery (Fig. 3).

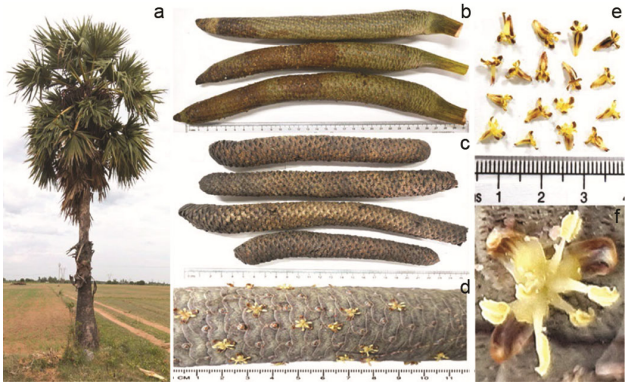

Fig. 2 — a) Habit, b) Fresh male inflorescence, c) Dried male inflorescence, d) Inflorescence with staminate flower, e) Dried flower, and f) Single flower enlarged.

Detailed TS of the central axis shows a single layer of vertically elongated wavy epidermal cells with brownish content followed by thin-walled parenchymatous ground tissue embedded with brownish content, idioblast cavities containing acicular crystals of calcium oxalate, conjoint collateral closed fibro-vascular bundles varying in size, peripheral fibre groups contain yellow pigment and smaller in size than the centre; vascular bundles consist xylem towards the centre encircled by phloem, an arc of sclerenchyma cells and bundle sheath cell towards the periphery; Detailed TS of the axis pith shows oval outline embedded with many male flowers, peripheral flower are matured, and immature flowers are towards the inner side; each flower consists of 3 sepals having a V shape outline and 3 petals having a crescent shape outline (Fig. 3).

Bracts

Detailed TS of the scale leaves (bracts) shows a single layer of epidermis embedded with brownish content and is covered by a thick cuticle; underneath 1 to 3 layers of collenchymatous hypodermis present

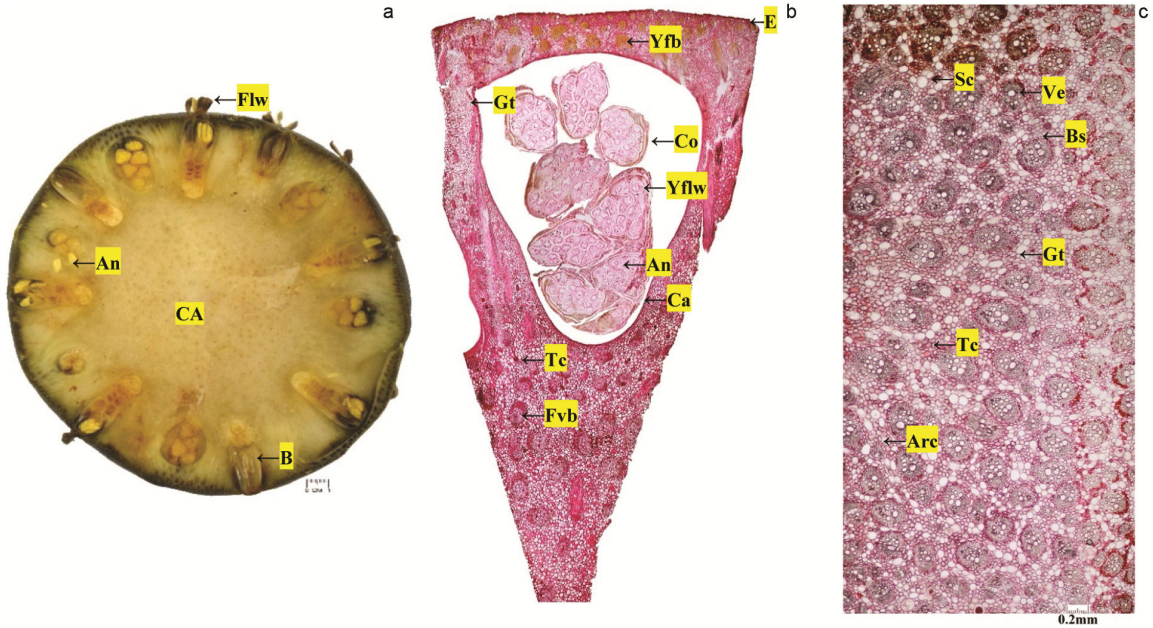

Fig. 3 — TS of *B. flabellifer* inflorescence axis, a) TS of inflorescence axis, b) Outer region enlarged, and c) Inner region enlarged. An - anther; Arc - acicular crystal; B - bract; Bs - bundle sheath cells; Ca - calyx; CA - central axis; Co - corolla; E - epidermis; Flw - flower; Fvb - fibro-vascular bundles; Gt - ground tissue; SC - secretory cavity; Tc - tanniferous cells; Yfb - yellow fibre bundle; Yflw- young flower; Ve – vessel.

followed by a wide zone of thin-walled parenchymatous ground tissue embedded with brownish content, clusters of thick-walled sclerenchymatous bundles, and few secretory cavities containing hazy sap with bundles of acicular crystals (Fig. 4).

Flower

Pedicle

TS of the flower stalks showed a wavy circular outline: A single layer of thin-walled epidermis followed by a wide zone of parenchymatous ground tissue embedded with cavities, brownish content, fibrovascular bundles, bundles of acicular crystals of calcium oxalate, and 2 or 3 locules at the centre (Fig. 5a).

Outer tepals

TS of outer tepals showed a curved shape on the adaxial side and V shape on the abaxial side; a single layer of the thick-walled epidermis containing brownish content and covered by thick cuticle, followed by 1 or 2 rows of collenchymatous hypodermis; round to oval, a thin-walled wide area of central parenchymatous pith in the adaxial side embedded with a thick-walled discontinuous group of the fibro-vascular bundle on the midrib region and a narrow continues line of sclerenchyma cells on both the curve ends (Fig. 5b).

Inner tepals

TS of inner tepals showed a crescent-shaped outline: The midrib region consisted of a single layer of upper and lower thick-walled epidermis containing brownish content and covered by a thin cuticle, followed by a collapsed, thin-walled, central parenchymatous pith embedded with brownish content, acicular crystal of calcium oxalate, and a thick-walled discontinuous group of fibrovascular bundles; taper to each end containing narrow continues line of thick-walled sclerenchymatous tissue (Fig. 5c).

TS of the anther showed a dithecous, longitudinally dehiscent anther wall; the pollen chamber contains many rounds to oval pollen grains, and the marginal portion of the section is embedded with brownish content and avascular bundle. The pollen grains consist of a granular aperture with a narrow, smooth membrane of exine, followed by a very narrow intine and pollen mother cell at the centre (Fig. 5d and e).

Powder microscopy

The powder shows epidermis cells in surface view with paracytic stomata; fragments of hypodermal cells; fragments of parenchyma cells with yellowish brown content, and a group of stone cells; fragments of anther endothecium cells, fragments of vessels with reticulate, spiral and annular vessels; fibres thin-walled with forked, pegged appearance, wide lumen and sharp end, a few thick-walled fibres with narrow lumen; a few tracheids with blunt end; pollen grains in surface view; simple starch grains, reddish brown content, tannins content, and acicular crystals (Fig. 6).

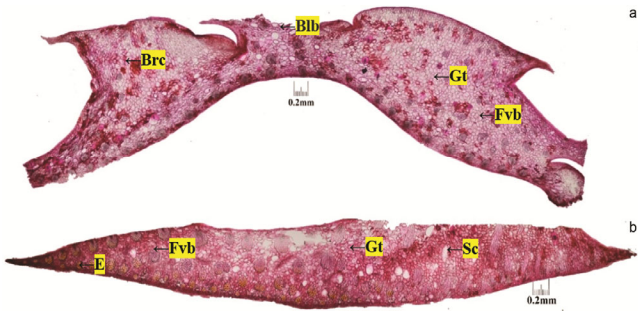

Fig. 4 — TS of *B. flabellifer* bracts a) Outer region enlarged, b) Inner region enlarged. Arc - acicular; Blb - bract leaf base; Brc- brownish content; E- epidermis; Fvb- fibro-vascular bundles; Gt- ground tissue; RaP- rachilla pith; SC – secretory cavity; Scl – sclerenchyma.

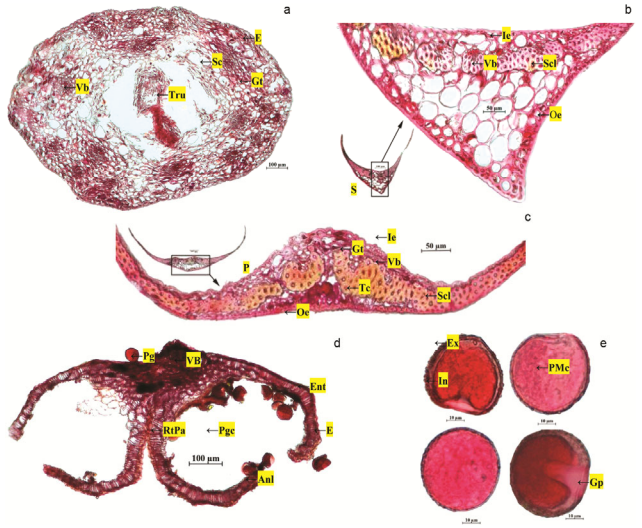

Fig. 5 — TS of *B. flabellifer* flower a) TS of pedicle, b) TS of outer sepal, c) TS of inner petal, d) TS of anther, and e) Pollen grains.Anl - anther lobes; E - epidermis; Ent - endothecium; E - epidermis; Ex - exine; Gp - germ pore; Gt - ground tissue; Ie - inner epidermis; It - intine; Oe - outer epidermis; P - petals; Pg - pollen grains; Pgc - pollen chamber; PMc - pollen mother cells; RtPa - reticulated parenchyma; S - sepals; Sc - secretory cavities; Scl - sclerenchyma; Tc - tanniferous cells; Tru - trabeculae; Vb - vascular bundle.

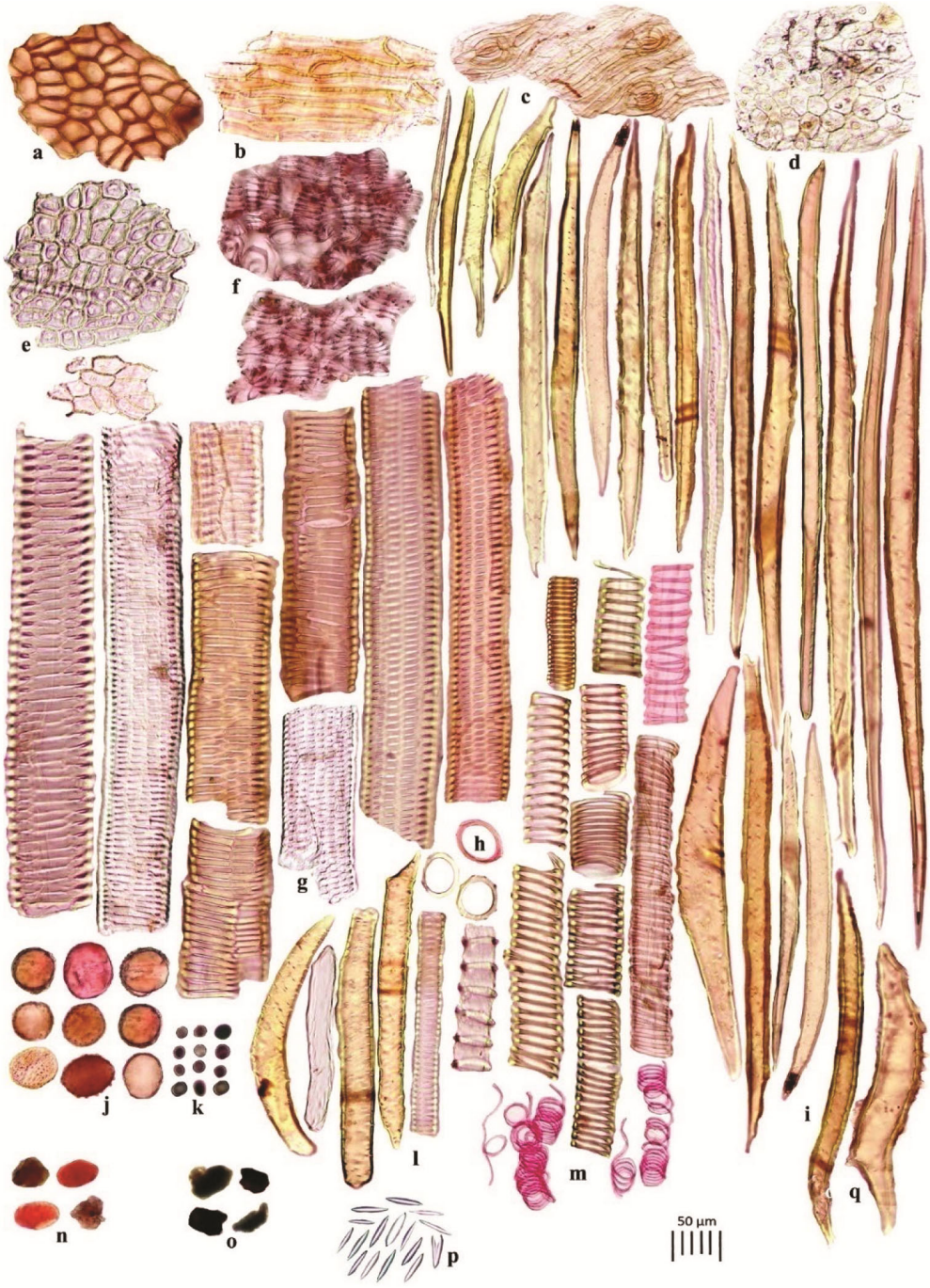

Fig. 6 — Powder Microscopy of *B. flabellifer* male inflorescence. a**)** fragment of the epidermis in surface view, b) fragment of hypodermal cells, c) epidermal cells with paracytic stomata, d) fragment of parenchyma cells with yellowish brown content, e) group of stone cells, f) fragment of anther endothecium cells, g) reticulate vessel, h) annular vessels, i) different shape of fibres, j) pollen grains in surface view, k) simple starch grains, l) tracheid, m) spiral vessels, n) reddish brown content, o) tannins content, p) acicular crystals and q) sclereids.

Physico-chemical tests

The results of various physico–chemical tests, namely of loss on drying, total ash, water-soluble extractive, acid-insoluble ash, alcohol-soluble extractive, water, and pH, are summarized in Table 1.

TLC/HPTLC fingerprinting

Densitometric study of the TLC plate of methanol extract of BfA, BfND and BfT showed 8, 6 & 5 bands respectively under short UV 254 nm (Table 2). Under long UV 366 nm 2, 1 & 2 spots bands were visible for

BfA, BfND and BfT respectively (Table 3). Post derivatized plate under white light revealed 12, 11 & 11 no of bands respectively for BfA, BfND and BfT (Table 4).

A densitometric scan at 254 nm showed 8, 6 and 5 peaks for BfA, BfND, and BfT, respectively. Scanning under this short UV, the peak of BfA at R_f 0.79 (area 41.68%), the peak of BfND at Rf 0.79 (area 56.52%) and the peak of BfT at R_f 0.79 (area 52.01%) emerged as the prominent for the respective samples (Fig. 7a). Fingerprinting at 366 nm revealed 2, 1, and 2 peaks for BfA, BfND, and BfT, respectively. Peaks with R_f 0.78 (area 94.16%), R_f 0.78 (area 100%) and R_f 0.78 (area 80.35%) became paramount for BfA, BfND and BfT respectively under this long UV scanning (Fig. 7b). Further scanning at 520 nm of the post derivatized plate divulged 12, 11, and 11 peaks for BfA, BfND, and BfT respectively. This profiling showed that BfA's peak at R_f 0.72 (area 39.43%), BfND's peak at R_f 0.72 (area 28.99%), and BfT's peak at R_f 0.72 (area 40.32%) were the most significant (Fig. 7c).

Table 1 — Physico-Chemical Parameters of *Borassus flabellifer*

Parameters (%)	BfA	Bf ND	BfT
Loss on drying	10.13	8.48	8.21
Total ash	3.84	6.60	2.97
Acid insoluble ash	2.36	1.00	1.37
Water soluble extractive	0.40	1.78	1.61
Alcohol soluble extractive	0.46	1.17	1.72
pH	5.72	5.69	5.10

Table 2 — TLC profile of *Borassus flabellifer at* 254 nm

BfA		BfND		BfT	
R_f	Colour	R_f	Colour	R_f	Colour
0.03	Green	0.02	Green	0.01	Green
0.09	Green	-	-	-	-
-	-	0.29	Green	0.29	Green
0.30	Green	-	-	-	-
0.43	Green	0.43	Green	0.43	Green
0.55	Green	-	-	-	-
0.65	Green	0.65	Green	0.65	Green
0.72	Green	-	-	-	-
0.79	Green	0.79	Green	0.79	Green
-	-	0.86	Green	-	-

Table 3 — TLC profile of *Borassus flabellifer* at 366 nm

BfA		BfND		BfT	
R_f	Colour	R_f	Colour	R_f	Colour
0.71	Blue	-	-	-	-
0.78	Fluorescent blue	0.78	Fluorescent blue	0.78	Fluorescent blue

DNA based authentication

As the market samples were exactly matching in their maco microscopic, physicochemical, and phytochemical profiling to the authentic drug, the DNA barcoding study was carried out for the field-collected authentic sample (BfA) and one of the market samples (BfND). PCR-based amplification of conserved region rbcL was used to establish DNA barcodes for species identification (Fig. 8). Nucleotide sequences were obtained from the chromatograms (Fig. 9). The primer resulted in an efficient amplification. The sequence homology of the amplified sequences was detected using the Basic Local Alignment Tool (BLAST). The sequence length was 856, and the BLAST similarity was 99.30%. The nucleotide sequence and the DNA Barcode generated are given in Table 5. The nucleotide sequences were deposited with BlankItid 2784886 and 2784896 and

Table 4 — TLC profile (white light) *Borassus flabellifer*

BfA		BfND		BfT	
R_f	Colour	R_f	Colour	R_f	Colour
-	-	-	-	0.01	
0.04	green	0.04	green	0.04	green
0.05	green	-	-	0.07	
-	-	0.12	green	0.12	green
0.13	violet	0.24	green	0.21	
0.26	violet	0.33	-	0.27	green
0.41	gray	0.40	gray	-	-
-	-	0.46	violet	0.46	violet
0.49	violet	-	-	-	-
-	-	0.52	violet	0.52	violet
0.53	violet	-	-	-	-
0.65	violet	0.64	violet	0.66	violet
0.72	green	0.72	green	0.72	green
0.79	green	0.77	green	-	-
0.81	violet	-	-	-	-
0.84	violet	0.84	violet	-	-
-	-	-	-	0.86	violet

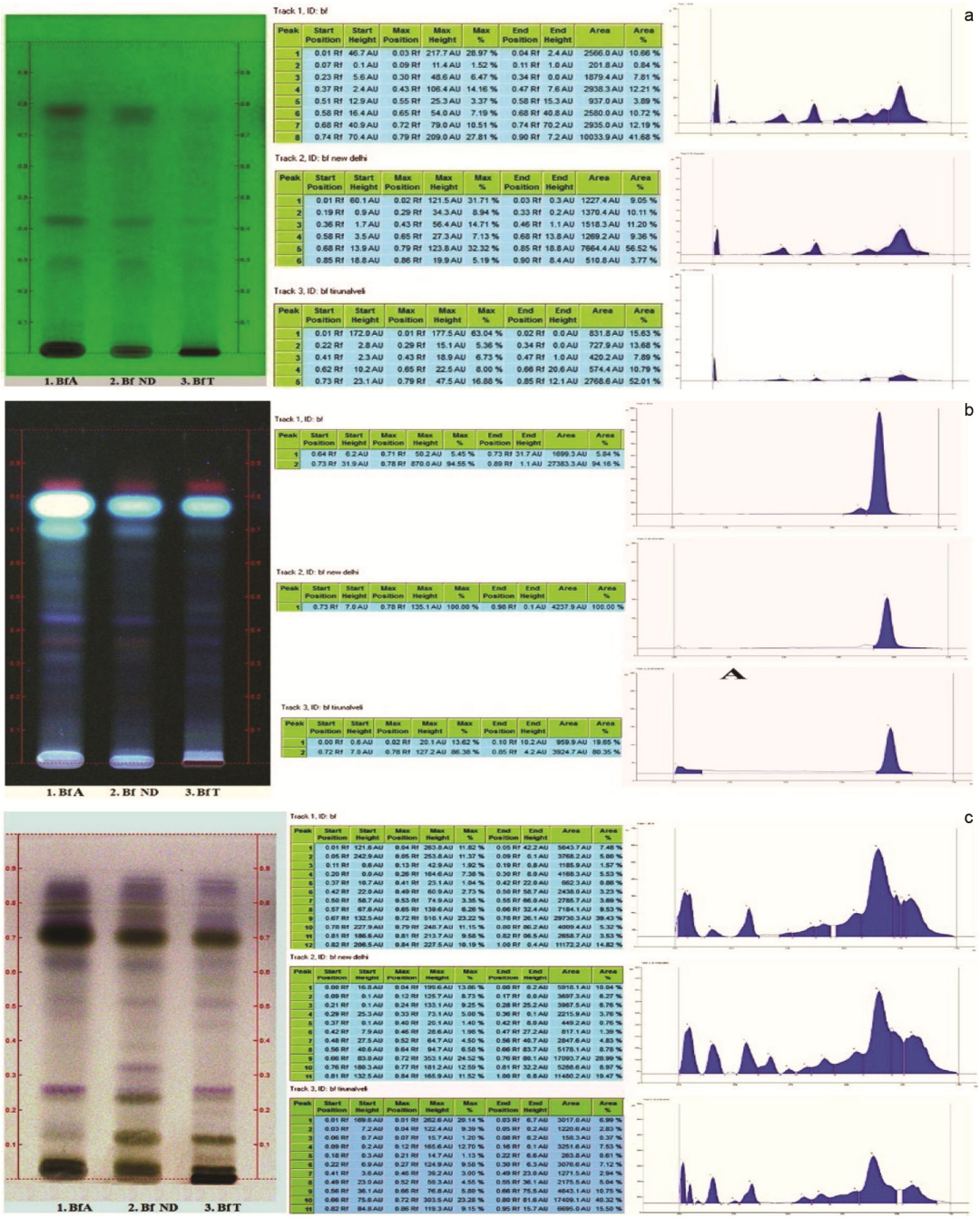

Fig. 7 — HPTLC densitogram of *B. flabellifer* at a) 254 nm, b) 366 nm, and c) 520 nm of *B. flabellifer* (Derivatized with VSR).

the accession numbers were obtained: PP107872 and PP1078723.

Discussion

Herbal drugs have morphological similarities, and when samples are in the form of dried crude drugs, it is often difficult to differentiate from each other, making identification of a sample difficult[21]. This raw material is different and has no relationship with the drug *Gajapippali*. Since the morphological features of medicinal plants are often not intact in the raw, studying the morphological, anatomical, chemical and DNA nucleotide sequences of their common substitutes might solve the problem of adulteration[22].

Macro-microscopy and chromatography techniques (TLC/HPTLC) are essential and crucial methods for identifying adulterants/substitutes in Siddha

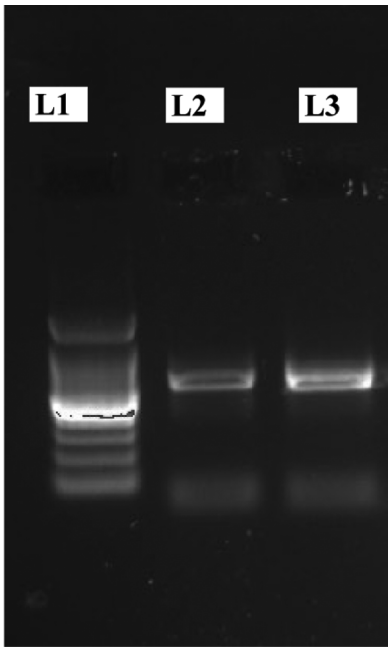

Fig. 8 — Gel image of PCR amplified product. L1: Ladder; L2: BfA; L3: BfND.

medications and also for quality control. Organoleptic characteristics, such as the presence, size, shape, colour, surface markings, edges (leaf), texture, fracture, internal appearance, cut surfaces, odour, and taste of the crude drug, are included in the macroscopic analysis. The identification of the type of cells, their contents, and their spatial organization within tissues are all included in the microscopical characters[23,24]. HPTLC, a technique where the authenticity and quality assurance of herbal drugs are frequently accomplished for the identification of specific compounds, can also be useful[25].

With powder microscopy, the appearance of essential features like acicular crystals, stone cells, spiral vessels, starch grains, and fibres might be helpful for further studies to differentiate contaminants present[26,27]. Quantitative characteristics such as the size of the epidermis, hypodermis, cortex cells, starch grains, the number and distribution of vascular bundles, and the presence or absence of specific cells can be documented by viewing the cross-sections of the samples[28]. The physicochemical parameters constitute one of the basic

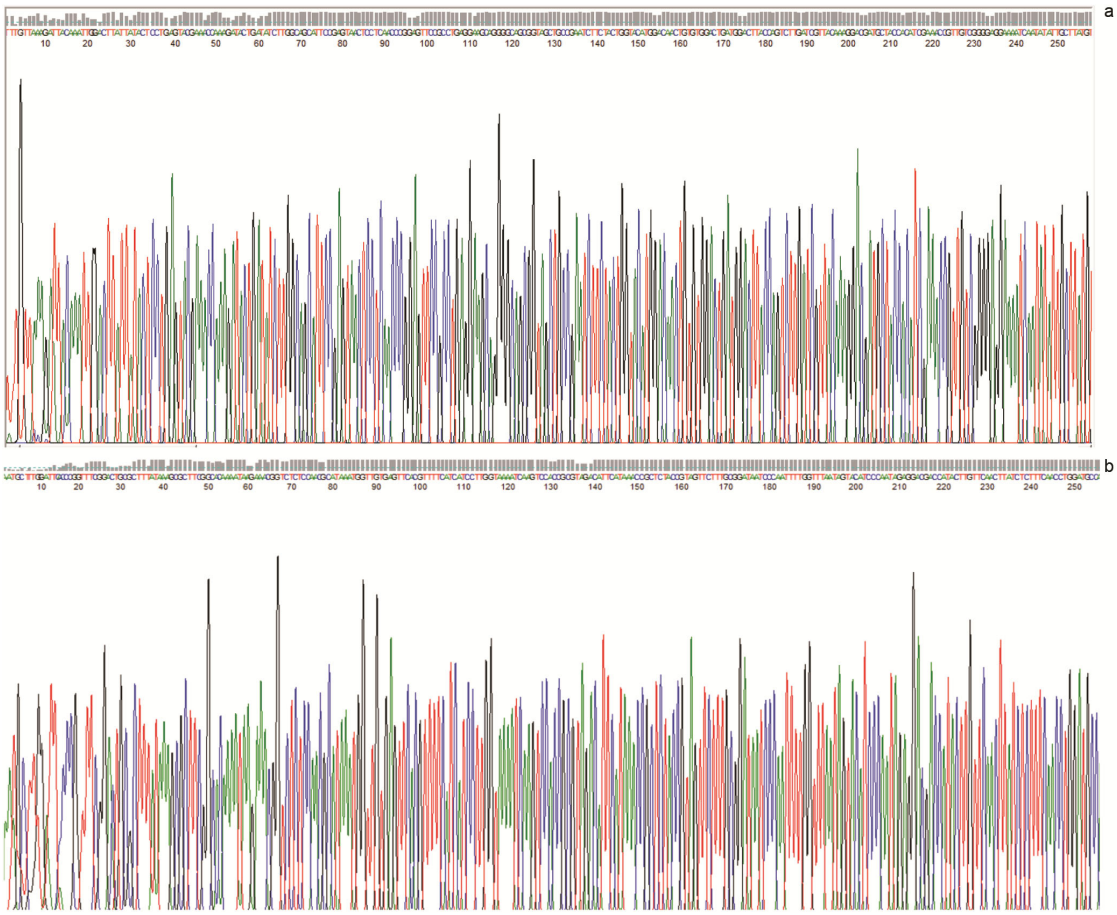

Fig. 9 — Chromatograms of *B. flabellifer* a) SCRIPCOGBF08 (BfA, and b) SCRIPCOGBF09 (BfND).

Table 5 — DNA Sequences and Barcode

Sample	Sequence and Barcode
SCRIPCOGBF08 (BfA)	Aatgctttggattcacccggtttcggactgcgctttataaagcgcttcggcacaaaataagaaacggtctctccaacgcataaatggttgtgagttcacgttttcatca tccttggtaaaatcaagtccaccgcgtagacattcataaaccgctctaccgtagttctttgcggataatcccaattttggtttaatagtacatcccaatagaggacgacc atacttgttcaacttatctctttcaacctggatgccatgaggcgggccttggaaagttttggaataagaaggggaattcgcagatcctccagacgtagagctcgtag ggctttgaaaccaaatacattacctacaatggaagtaaacatgttagtaacagaaccttcctcaaaaaggtctaaaggataagctacaaagcaatatattgattttcct ccccgacaacggtttcgatgtggtagcatcgtctttgtaacgatcaagactggtaagtccatcagtccacacagttgtccatgtaccagtagaagattcggcagct accgctgcccctgcttcctcaggcggaactccgggttgaggagttactcggaatgctgccaagatatcagtatctttggtttcgtactcaggagtataataagtcaatt tgtaatctttaacaccagctttaaatccaacacttgctttagtttctgtttgtggggacatacaaaagctcgcatgtacctgcggtagctagtttcggttggtggtgacat agctcgcatgtacctgcaagagctgtttctggtgtggggtgcaaaaaatacccgcaggcgcccgagaacccccgggagaggggagggggtgaaaa
SCRIPCOGBF09 (BfND)	aatgctttggattcacccggtttcggactgcgctttataaagcgcttcggcacaaaataagaaacggtctctccaacgcataaatggttgtgagttcacgttttcatcat ccttggtaaaatcaagtccaccgcgtagacattcataaaccgctctaccgtagttctttgcggataatcccaattttggtttaatagtacatcccaatagaggacgacc atacttgttcaacttatctctttcaacctggatgccatgaggcgggccttggaaagttttggaataagaaggggggaattcgcagatcctccagacgtagagctcgtag ggctttgaaaccaaatacattacctacaatggaagtaaacatgttagtaacagaaccttcctcaaaaaggtctaaaggataagctacataagcaatatattgattttcct ccccgacaacggtttcgatgtggtagcatcgtctttgtaacgatcaagactggtaagtccatcagtccacacagttgtccatgtaccagtagaagattcggcagct accgctgcccctgcttcctcaggcggaactccgggttgaggagttactcggaatgctgccaagatatcagtatctttggtttcgtactcaggagtataataagtcaatt tgtaatctttaacaccagctttaaatccaacacttgctttagtttctgtttgtggggacatacaaaagctcgcatgtacctgcggtagctagtttcggttggtggtgacat agctcgcatgtacctgcaagagctgtttctggtgtggggtgcaaaaaatacccgcaggcgcccgagaacccccgggagaggggagggggt

studies for herbal raw drugs. The loss drying gives the total content of moisture and volatile oil. All three samples of *B. flabellifer* showed the value for loss on drying below 11 per cent. The total ash of the three samples was 3.84, 6.60, and 2.97, respectively, which represents the inorganic salts required for the physiological functions of the human body. The acid insoluble ash for the samples were 2.36 (BfA), 1.00 (BfND), and 1.37 (BfT), respectively. The alcohol-soluble and the water-soluble extractives are indicative of the solubility of the secondary metabolite constituents in the high polar organic and inorganic solvents, which was least in the BfA compared to the other two samples. The pH in the range of 5.10 to 5.72 indicates the acidic nature of the drug. The methanol extractive indicates the presence of high polar compounds.

Densitometric observations under UV 254 nm revealed 3 common bands with R_f 0.43, 0.65, and 0.79, respectively, for all the samples. Band with R_f 0.78(blue) appeared as equivalent for all three samples under UV 366 nm. Bands with R_f 0.04(green) and 0.72(green) seemed to be identical for all three samples under white light.

The significant peaks identified during short UV scanning for BfA, BfND, and BfT were observed at R_f values of 0.74 (area 41.68%), 0.68 (area 56.52%), and 0.73 (area 52.01%), respectively. In contrast, during long UV scanning, the prominent peaks for BfA, BfND, and BfT were recorded at R_f values of 0.73 (area 94.16%), 0.73 (area 100%), and 0.72 (area 80.35%). Furthermore, under white light scanning of the post-derivatized plate at UV 520 nm, the primary peaks for BfA, BfND, and BfT were found at R_f values of 0.67 (area 39.43%), 0.66 (area 28.99%), and 0.66 (area 40.32%), respectively.

Since DNA markers are notage-dependent, tissue-specific, and have better discriminatory power than the other two markers, they are preferred over them. DNA markers use nucleotide sequences to identify species. Therefore, identifying plants using these markers is the best method for identifying medicinal plant species, populations, and variants. DNA barcoding is an approach for identifying species that makes use of a small DNA fragment from a particular gene or genes. Every species has a unique barcode, just like every individual has a unique fingerprint. These DNA barcodes can be identified by comparing them to a reference library[29]. Compared to conventional morphological identification, DNA barcoding is useful for identifying medicinal plant species for conservation and application since it may confirm a genuine product rather than a substitute[30].

DNA barcoding in plants frequently uses nuclear DNA (ITS and ITS2 sections) and plastid DNA (rbcL, matK, trnL, and trnH-psbA regions)[31,32]. DNA markers use nucleotide sequences to identify species. Therefore, identifying plants using these markers is the best method for identifying medicinal plant species, populations, and variants[33]. The term "basic local alignment search tool," or "BLAST," refers to a group of software applications that are used to create alignments between sequences of nucleotides or proteins[34,35].

Molecular markers were preferred due to their reliability, consistency, and reduced susceptibility to environmental influences in comparison to chemical constituents and physical traits[36]. The findings of the current study suggest that the various universal primers used for DNA barcoding, rbcL is reliable for the amplification, identification, and determination of the male inflorescence of *B. flabellifer* with success rates of amplification of more than 90%. The primer resulted in very sharp bands, which made sequencing feasible.

These records would further help study the taxonomy, ecology, phylogeny, and morphology of different species. However, by displaying more precise genomic information from other species, the creation of new procedures and amplification techniques using novel primer combinations will drastically transform the field of DNA barcoding[20].

There is minimal availability on the morphological identification of the original drug source of *Gajapipali*, *S. officinalis*[37]. As a result, an immense amount of adulteration occurs. Previous literature has shown the presence of other species like *Piper chaba* due to its morphological similarities, *Balanophora fungosa* due to its superficial similarities, and *Pothos scandens* are sold in the market as the adulterants[38], but in the current study, we came across *Piper retrofractum* and *Piper mulleusa*. Due to insufficient and contradictory literature on the morphology of the plant, the identification is mistaken most of the time, which results in adulteration.

Adulteration has become a very prevalent practice in the herbal raw drug market. The pharmacognostical study helps in the standardization and authentication of true drugs[39]. In the current study, the market samples of *Gajapippali* were collected and subjected to macro microscopy, physicochemistry phytochemistry, and DNA Barcoding. The comprehensive study showed that the male inflorescence of *B. flabellifer* is being marketed in the name of *Gajapipali*. The genuine drug is the female inflorescence of *Scindapsus officinalis* which is used in the traditional medicinal systems for curing ailments. However, the adulteration of the drug with another herbal source lacking the same bioactivities and medicinal properties will lead to serious health implications.

Conclusion

The study aimed to check the authenticity of the market-traded samples of one of the important ASU drugs *Gajapipali*. The macro microscopical analysis, together with the chemical fingerprinting, showed the samples to be not the genuine drug *S. officinalis* but an admixture of several unrelated taxa with the male inflorescence of *B. flabellifer* forming the major share, which was finally confirmed by DNA Barcoding. It is high time that we look into the bioactivities of the marketed drug since they may lack the same bioactivities and medicinal properties as the genuine drug, and consumption of which will lead to serious health implications. This study will also prove to be a monograph of *B. flabellifer* for future reference.

Conflict of interest

The authors declare that they do not have any conflict of interest.

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