

Pharmacognostic, phytochemical, and chromatographic fingerprinting of three probable species accepted as *Kakoli* – a member of *Astavarga*

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Received 06 July 2021; revised 20 April 2022; accepted 11 June 2024

Kakoli is an Ayurvedic drug, used for its effect on the reproductive system, especially for promoting spermatogenesis. According to Ayurvedic and other Sanskrit literature, three different plant species exist under the name of *Kakoli*, namely, *Roscoea purpurea* Sm., *Roscoea capitata* Sm., and *Roscoea alpina* Royle. Grouping multiple plant species under one name, often leads to the selection and inconsistent usage of non-authentic species as therapeutic drugs. In the present study, we compared the three *Roscoea* species, used as *Kakoli*, using pharmacognostical and phytochemical, as well as other analytical studies. We found that *R. purpurea* Sm. might be the most probable source of *Kakoli* as evidenced by the correlation obtained between literary resource-based parameters (ancient scriptures) and the experimental analysis (pharmacognostical and phytochemical findings) thereby establishing the authentic taxonomic identity of the valuable ancient medicine *Kakoli*.

Keywords: Ayurveda, *Kakoli*, *Roscoea purpurea* Sm., *Roscoea capitata* Sm., *Roscoea alpina* Royle, Spermatogenesis

IPC Code: Int Cl.²⁴: A61K 36/00

Ayurveda is one of the oldest medical sciences, which has been practiced since the fourth century BC^{1,2}. The basis of Ayurveda is the adoption of preventive and health-promoting practices in life. Herbal medicines have been identified as a main source of primary healthcare, with about four-fifths of the world's population still being dependent on traditional medicines³. The Indian subcontinent is a hotspot for medicinal plant species, with about 15,000 plant species utilised in the treatment of different diseases^{4,5}. However, the botanical identity of certain species is still ambiguous as there are several drugs from different plant species being sold under a single name. Furthermore, several medical practitioners also use substitutes or adulterants for a given Ayurvedic species and, over time, these alternative plant species also unintentionally acquire the same Sanskrit name. This ultimately leads to the misidentification or inappropriate usage of medicinal plant species^{6,7}.

One such example of a controversial classical drug plant is *Kakoli*, which is recognized as one of the *Astavarga* plants, a group of eight rare medicinal plants⁸. It has also been acknowledged in the *Caraka* and *Susruta Samhita* under the *Shukrajanana* (a group

of 10 medicinal plants used as spermatogenic drugs); *Snehopaga* (a group of 10 medicinal plants used as emollients); *Angamarda prashmana* (a group of 10 medicinal plants used as analgesics); *Jivaneeya Dashemaani* (a group of 10 medicinal plants used as rejuvenants and a tonic); and *Kakolyadi Varga* (a specific group of medicinal plants used for rejuvenation). A description of *Kakoli*, along with its Sanskrit synonyms, its habit, habitat, and medicinal properties, has also been mentioned in different *Nighantus*. In the absence of appropriate taxonomic data, three plant species have been identified as *Kakoli*, namely, *Roscoea purpurea* Sm., *Roscoea capitata* Sm., and *Roscoea alpina* Royle [Family: Zingiberaceae]^{9,10}.

The present study provides a comparison of the three plausible botanical species widely accepted as *Kakoli*. Our studies reveal that, although the botanical sources of *Kakoli* may differ, the basic pharmacognosy and phytochemistry are consistent.

Kakoli – Vitalizing herb of *Astavarga*

Kakoli is a medicinal herb designated to a group of eight medicinal plants (the *Astavarga* group) in accordance with the description of *Ācārya Śārangadhara*. Other members of the *Astavarga* group are *Kṣīrakākolī* (*Lilium polyphyllum* D. Don);

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Medā (*Polygonatum verticillatum* (L.) All.); *Mahāmedā* (*Polygonatum cirrhifolium* (Wall.) Royle); *Jīvaka* (*Crepidium acuminatum* (D. Don) Szlach.); *Riṣbhaka* (*Malaxis muscifera* (Lindl.) Kuntze); *Riddhī* (*Habenaria intermedia* D. Don); and *Vṛiddhī* (*Habenaria edgeworthii* Hook. f. ex Collett). Several Sanskrit as well as vernacular names has been given for *Kakoli*, as shown in Supplementary Table S1⁹.

In ancient literature, *Kakoli* has been mentioned as a holistic remedy for *Vaksogata roga* (thoracic diseases); *Udaragata roga* (abdominal diseases); *Vrkkavastigata roga* (renal & urinary bladder diseases); *Asthisamdihigata roga* (musculo-skeletal diseases); *Tvacagata roga* (dermatological diseases); *Manasa roga* (neurological disorders); *Sarvasariragata roga* (generalised body disorders); *Rasayana Vajikarana* (rejuvenation & virility strengthening); *Balaroga* (paediatric diseases); and *Visa chikitsa* (alexipharmic treatment). Details of these ailments are presented in Table 1. *Kakoli* has also been mentioned in modern research literature for its holistic medicinal utility⁹. The main therapeutic claims for *Kakoli* include its effectiveness as a nutritious tonic and aphrodisiac. It is also used for the management of polydipsia, sexual frigidity, fever, diabetes, and bone fracture^{8,9}.

Herbals proposed as *Kakoli* – Botanical identity of *Kakoli*

Several authors have presented varied views on the botanical identification of *Kakoli*. The first mention of *Astavarga* plants was found in a book named ‘*Dravyaguna Vijnana*’, authored by Acharya Priyavrat Sharma (1954); however, a detailed description of *Kakoli* was absent¹¹. In the modern era, Dr. Krishna Chandra Chunekar first attempted to give

a botanical description of *Kakoli* under the names *Roscoea procera* Wall. (Syn. *Roscoea purpurea* Sm.) and *Roscoea alpina* Royle¹². Several other authors were of the same opinion; however, others put forward alternative views that *Roscoea capitata* Sm., *Fritillaria cirrhosa* D. Don, and *Lilium polyphyllum* D. Don were probable sources of *Kakoli*. The views of modern scholars on the probable botanical identity of *Kakoli* are presented in Supplementary Table S2. According to the ancient scriptures, i.e., *Shaligram* and *Bhav Prakash Nighantu*, the existence of purple-coloured flower and stout stem are considered as primary identification feature of *Kakoli*. Till date no absolute attempt has been made towards the correct identification of *Kakoli*, and there exists much ambiguity about its most possible botanical names. Henceforth, the current research focuses on the precise identification of *Kakoli* by comparing the botanical description of *Kakoli* as portrayed in several *Nighantus* and *Samhitas* with the its taxonomical and botanical explanations as described in various modern floras. Upon comparing the Ayurvedic and other Sanskrit literature with the modern botanical features as provided in the floras, three different plant species were found to show maximum similarity with *Kakoli*, namely, *Roscoea purpurea* Sm., *Roscoea capitata* Sm., and *Roscoea alpina* Royle. In the ensuing sections, the authors will be describing the probable sources of *Kakoli* (*Roscoea purpurea* Sm., *Roscoea capitata* Sm., and *Roscoea alpina* Royle) for establishing the actual identity of *Kakoli*.

Roscoea purpurea Sm. as *Kakoli*

Roscoea purpurea Sm. is a robust plant with thick roots and pale lilac to mauve, but also purplish- pink

Table 1 — Ethnomedicinal uses of *Kakoli* as mentioned in ancient Indian scriptures

S. No.	Category of Disease	Specific conditions treated by <i>Kakoli</i>
1	<i>Vaksogata roga</i> (Thoracic diseases)	<i>Urahsakata</i> (Chest injury), Pulmonary haemorrhage, Tuberculosis, <i>Kasa</i> (Cough), <i>Svasa</i> (Asthma) & Bronchitis
2	<i>Udaragata roga</i> (Abdominal diseases)	<i>Gulma</i> , Diarrhoea, Dyspepsia
3	<i>Vrkkavastigata roga</i> (Renal & urinary bladder diseases)	<i>Mutrakrcchra</i> (Dysuria)
4	<i>Asthisamdihigata roga</i> (Musculo-skeletal diseases)	<i>Vatarakta</i> (Gout)
5	<i>Tvacagata roga</i> (Dermatological diseases)	<i>Nari vrana</i> (Sinus), Boils & Stomatitis
6	<i>Manasa roga</i> (Neurological disorders)	<i>Unmada</i> (Insanity)
7	<i>Sarvasariragata roga</i> (Generalised body disorders)	Anaemia, Malaria, Paresthesia (Burning sensation), Remittent fever & General debility
8	<i>Rasayana Vajikarana</i> (Rejuvenation & Virility strengthening)	Oligospermia (Low sperm count) & <i>Sukra daurbalya</i> (Sexual debility), <i>Roganasaka</i> (Immunomodulator), <i>Balya</i> (Tonic)
9	<i>Balaroga</i> (Paediatric diseases)	Medicine for Child emaciation
10	<i>Visa chikitsa</i> (Alexipharmic treatment)	Scorpion & Spider poison antidote

(*R. purpurea* Sm. 'Vincent'), white, and rarely red (*R. purpurea* Sm. 'Red Gurkha') flowers¹³. This plant was first described taxonomically by James Edward Smith in 1808, based on Scotsman Francis Buchanan specimens procured from Nepal¹⁴. This species is indigenous to the Himalayas, ranging from Himachal Pradesh to Assam (India) and also to Nepal and the Bhutan at an altitude ranging between 1,500-3,200 m above sea level. *Roscoea purpurea* Sm. has thick, rhizomatous root system. Leaves are four to eight in number, 12-20 cm long, soft, green colored, smooth or ciliated, with leaf blades horizontal to recurved. Its leaf sheaths are often purple or red colored, and narrow bracts are ovate and typically covered by the upper leaves. The flowers appear from the month of June to September in succession to the apical leaves and are purple, mauve, red, or white colored¹⁵. It is cultivated as an ornamental species and, in northern India. Its fleshy roots are traditionally used as tonic to treat malaria, sexual frigidity, and also as remedy against hematemesis, excessive thirst, and rheumatic pain⁸.

Roscoea capitata* Sm. as *Kakoli

Roscoea capitata Sm. is a rare Nepalese plant with light pink-purple flowers (occasionally pale white), borne in a tight head, held well above the leaves¹⁵. This plant was first described taxonomically by James Edward Smith in 1822. *Roscoea capitata* Sm. grows wild in central Nepal and is a member of the ginger family (*Zingiberaceae*)¹⁴. In Nepal, it is confined to a small area north-west of Kathmandu. It grows typically at 1,200-2,600 m above sea level. It has tuberous roots which emerge from a fleshy rhizome. The pseudostems (formed from sheathing leaves) are up to 45 cm tall. There are three to nine leaves, which are soft and fleshy, curving and somewhat wavy, bright green and smooth or with a fringe of hairs along the margin. *Roscoea capitata* Sm. is the only Nepalese species of the genus that holds its inflorescence above the leaves on a peduncle. Flowering in this species occurs during the monsoon season (June to September). Each orchid-like flower lasts for only one or two days. The staminodes of the flowers are characteristically long, almost the same length as the dorsal petal^{15,16}. The rhizomes of this ornamental species are edible and often used as an aphrodisiac¹⁷.

Roscoea alpina* Royle as *Kakoli

Roscoea alpina Royle is a perennial, herbaceous plant species, considered native to the eastern to

western Himalayas comprising countries like India, Pakistan, Nepal and Bhutan. The species was first described taxonomically by John Forbes Royle in 1839¹⁴. *Roscoea alpina* Royle is perennial, terrestrial, herb with fleshy, rhizomatous and tuberous roots. This plant is about 10-18 cm tall, with four to six, 7.62-10.16 cm long leaves. First two to three leaves comprises sheath, while the later contains glabrous leaf blade. A small ligule, about 0.5 mm is present at the intersection of the leaf blade and sheath. *Roscoea alpina* Royle flowers are deep purple or lilac, with flowering occurring between May and August¹⁵. This species has often been used as a remedy for sexual frigidity¹⁷.

Materials and Methods

Collection and processing of plant material

Roscoea purpurea Sm., *R. capitata* Sm., and *R. alpina* Royle were collected from Uttarakhand state (latitude and longitude range: 30.0668° N, 79.0193°E). All the collected herbals were authenticated by the authors, with a specimen deposited in the repository of Patanjali Research Institute, Haridwar [voucher specimens No PRFH 2259, PRFH 2261 and PRFH 2252, respectively]. The shade-dried rhizome and stem of all species were ground mechanically and then filtered by using 125 size sieve, before being analysed. Amorphous yellowish powder was obtained in each case. Aliquots of the powder were stored in airtight containers for conducting the experimental procedures (solubility tested in ethanol, water, and 1:1 water: alcohol)¹⁸.

Plant raw material identification

Characterization of the raw herbal samples was accomplished by microscopic examination. Microscopic identification was done by analysing transversely and longitudinally cut sections of rhizome and stem under field emission scanning electron microscopy (FE-SEM, Model HV-EXP796, HoverLabs, 2018, India). The powdered material was also examined under the microscope¹⁹.

Herbal powder characterization

Characterization of the powdered herbal materials followed the standardised WHO guidelines for the identification of ash content, acid-insoluble ash content, ethanol-soluble extract content, moisture content, water-soluble extract content, and pH value²⁰.

Phytochemical analysis

Quantitative analysis of polyphenol, flavonoid, saponin, and proanthocyanidin content was done by

spectrophotometric analyses. Polyphenol content was measured by mixing 10 g powdered herbal sample in a mixture of 50 mL of distilled water and 5 mL of Folin Ciocalteu reagent (10% v/v). After this, 4 mL of 7.5% w/v sodium carbonate was added to the mixture, followed by homogenisation and incubation at 25°C for 2 h. The solution absorbance was measured using a spectrophotometer at 760 nm. Gallic acid was used as the standard, and the results were expressed as gallic acid equivalent²¹.

The total flavonoid content was investigated by colorimetric assay using aluminium chloride. The reaction mixture comprised 1 mL of powdered herbal material and 4 mL of distilled water; 0.3 mL of 5% w/v sodium nitrite was supplemented and, after 5 min, a further 0.3 mL of 10% w/v aluminium chloride was added. After 5 min, 2 mL of 1 M sodium hydroxide was added, and the reaction mixture was diluted to 10 mL with distilled water. A set of reference standard solutions of quercetin was processed by previously described method for the test samples. A UV/Visible spectrophotometer was used with the reagent blank at 510 nm wavelength for determination of absorbance for test and standard solutions. The total flavonoid content of the extract was determined in mg of QE/g (Quercetin Equivalent)^{22,23}.

The total saponin content of the herbal samples (1 mg/mL) was measured by adding vanillin reagent (8% w/v, 0.25 mL) and sulphuric acid (72% v/v, 2.5 mL) to the samples. This mixture was cooled in ice-cold water for 10 min, after which the absorbance was measured at 544 nm. Diosgenin solution was used as the standard. Proanthocyanidin estimation was also done by mixing the herbal samples (1 mg/mL) with 3 mL vanillin and 1.5 mL hydrochloric acid. The absorbance was measured at 500 nm by using catechin as the standard²⁴.

Chromatographic and spectroscopic analysis

High Performance Thin Layer Chromatography (HPTLC)

The extracted herbal samples (2 g) were individually extracted in a methanol: water (9:1) mixture and analysed on 20 × 10 cm high performance thin layer chromatography (HPTLC) 60F254 pre-coated silica gel plate [washed using methanol~ 20 mL/ trough in a 20 × 10 cm twin-trough chamber and dried at 120°C, followed by equilibration in a solvent-phase-saturated chamber]. The filtered sample (filter size: 0.45 µm, volume limit: 20 µL) was injected using a Hamilton syringe-

mediated automated injector system (Linomat IV, Camag, USA). Chromatographic separation of *R. capitata* Sm., *R. procera* Wall., and *R. alpina* Royle was carried out for 20 min at 37°C using the mobile phase as chloroform: acetone: diethylamine (70:20:10); toluene: acetone: glacial acetic acid (100:3:0.07); and benzene: ethyl acetate: glacial acetic acid (90:5:5), respectively. The visualization of developed TLC plates were performed at the absorption λ of 254 and 366 nm in the TLC scanner (Camag, USA). Mescaline, ellagitannin, and gallic acid were used as standards for comparative R_f values^{25,26}.

Liquid chromatography mass spectrometry

The raw extract (2 g) was dissolved in a mixture of 10 mL methanol: water (9:1) v/v and sonicated, which was further analysed on a Waters HPLC system, using a Sunfire C18 column with auto-injector and diode array detector. Elution was carried out at a flow rate of 1 mL/minute with the mobile phase as 0.1% w/v orthophosphoric acid in water with diethylamine. Equilibration time was 55 min with a 7-step gradient of geometric progression. Liquid chromatography–mass spectrometry (LC-MS) analysis was performed using an LCQ MS mass spectrometer. The comparative analysis of eluted peak data of the powdered herbal samples was done using the mass spectral data of standard compound(s), namely, gallic acid, catechin, caffeic acid, and quercetin²⁷.

Correlation matrix

A comparative analysis was done, based on various physical, morphological, and therapeutic claims for *Kakoli* (as mentioned in ancient scriptures) and the three probable sources, namely, *Roscoea purpurea* Sm., *R. capitata* Sm. and *R. alpina* Royle. The experimentally deduced standardized parameters for the three species were then used as the criteria to reach a decision regarding the botanical identity of *Kakoli*²⁸.

Results and Discussion

Microscopic analysis of raw material

Roscoea purpurea Sm.

Rhizome: The endodermis was single-layered and irregularly arranged; the pericycle was one to two-layered; and the vascular bundles were radially arranged, exarch, numerous, and scattered. Polyarch arrangements of vascular bundles were observed, with

phloem strands often extended radially inwards. Xylem vessels were arranged alternately with phloem patches; xylem vessels were solitary and showed spiral thickening; and xylem consisted of xylem fibers, vessels, and tracheids. The pith was made of oval, parenchymatous cells (Fig. 1a).

Stem: The endodermis was one to two-layered; the pericycle was single-layered; and the vascular bundles were endarch in nature. The metaxylem was oriented towards the stem periphery, and the protoxylem point was arranged towards the pith. Vascular bundles present, four to six in number; xylem consisted of tracheids, vessels and parenchyma cells; phloem consisted of sieve cells, companion cells and fibers; and the parenchymatous cells were filled with

numerous oval to elliptical starch grains. The pith was composed of small, parenchymatous cells (Fig. 1b).

Powder: The powder was yellow in colour: cortical cells were filled with brown dark-coloured materials; starch grains were both simple and compound and present in many cortical cells. Cork cells, in surface view, showed several fragments of cortical parenchyma cells, stone cells in groups, secondary xylem with pitted vessels, and phloem cells (Fig. 1c).

***Roscoea capitata* Sm.**

Rhizome: The endodermis was single-layered, with thin-walled cells; indistinct pericycle; and crescent-shaped vascular bundles which were arranged in two arches. Xylem vessels were arranged

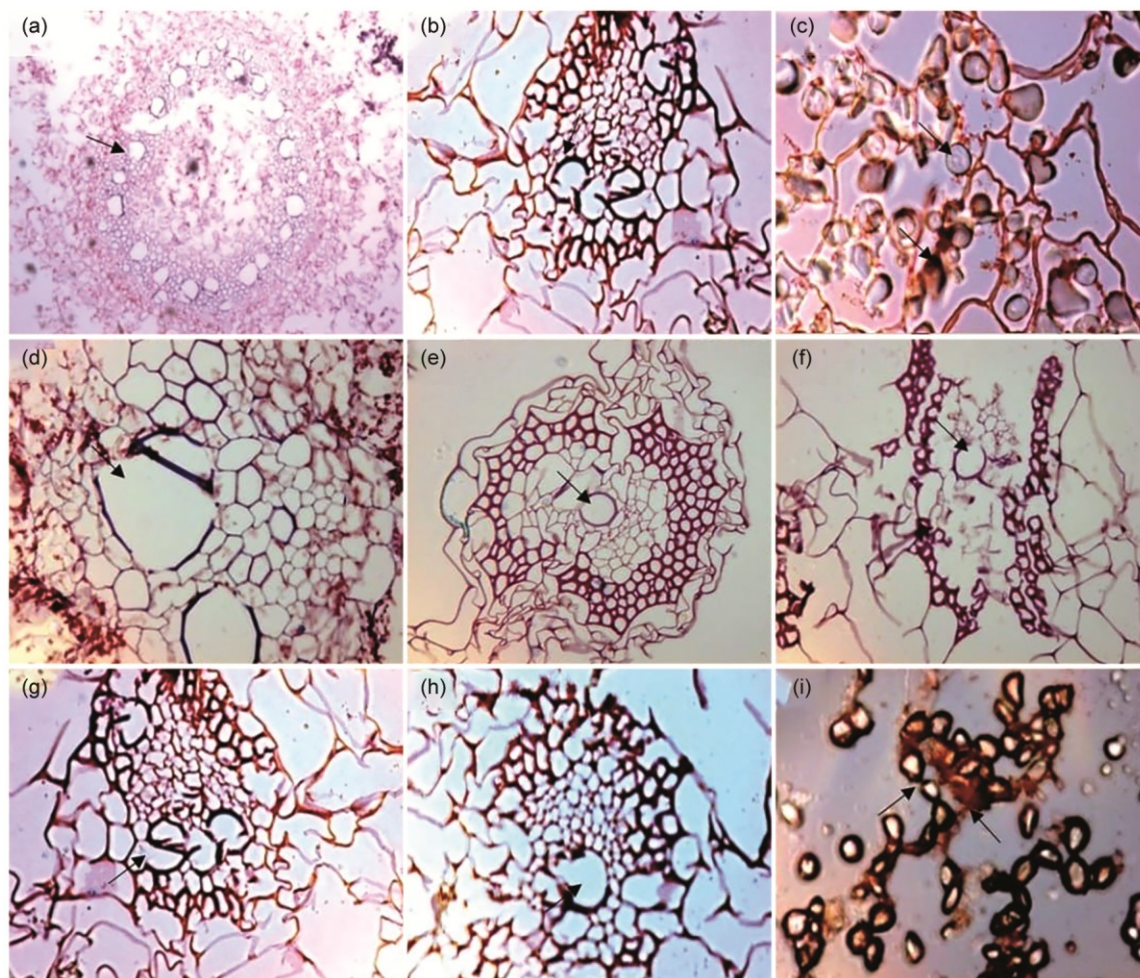


Fig. 1 — Microscopic analysis of rhizome, stem and herbal powder. (a) Rhizome of *Roscoea purpurea* Sm. — radially arranged vascular bundle; (b) Stem of *Roscoea purpurea* Sm. — oval to elliptical starch grains; (c) Powder of *Roscoea purpurea* Sm. — oval to polygonal, sieve cells and dark coloured substances; (d) Rhizome of *Roscoea capitata* Sm. — large, oval-to-polygonal pith cells, parenchymatous in nature; (e) Stem of *Roscoea capitata* Sm. — oval to elliptical starch grains; (f) Powder of *Roscoea capitata* Sm. — oval to polygonal sieve cells; (g) Rhizome of *Roscoea alpina* Royle — crescent shaped vascular bundle; (h) Stem of *Roscoea alpina* Royle — oval to polygonal starch grains; (i) Powder of *Roscoea alpina* Royle — oval to polygonal sieve cells and dark coloured substances

alternately with phloem patches, were solitary, and showed spiral thickening. Xylem consisted of xylem fibers, vessels, and tracheids. The pith comprised large, oval to polygonal parenchymatous cells, which contained starch grains (Fig. 1d).

Stem: The endodermis was single-layered; the pericycle was indistinct; and the vascular bundles were endarch in nature. Metaxylem was oriented towards the periphery, with the protoxylem points arranged towards the pith. The xylem comprised of parenchyma cells, tracheids and vessels; the phloem consisted of sieve cells, companion cells, and fibers; and the parenchymatous cells were filled with numerous oval to elliptical starch grains. The pith was composed of large, oval parenchymatous cells (Fig. 1e).

Powder: The powder was yellowish in colour and consisted of xylem fibers, fragments of parenchyma cells with starch grains, sclerenchymatous cells, and vessels. Starch grains had many, oval to polygonal, sieve cells and dark-coloured substances (Fig. 1f).

Roscoea alpina Royle

Rhizome: The endodermis was single-layered, with thin-walled cells; the pericycle was indistinct; and the vascular bundles were crescent-shaped and arranged in two arches. The xylem vessels were arranged alternately with phloem patches, were solitary, and showed spiral thickening; the xylem consisted of xylem fibers, vessels, and tracheids. The pith comprised large, oval to polygonal parenchymatous cells, containing starch grains (Fig. 1g).

Stem: The endodermis was single-layered; the pericycle was either indistinct or absent; and the vascular bundles were scattered radially and were endarch in nature. The metaxylem cells were oriented towards the periphery, and the protoxylem pointed towards the pith; xylem vessels were arranged alternately with phloem patches and were solitary with spiral thickening; the xylem consisted of xylem fibers, vessels, and tracheids. The pith was made up of oval, parenchymatous cells (Fig. 1h).

Powder: The powder was yellowish in colour and consisted of xylem fibers and fragments of parenchyma cells with starch grains. The starch grains had many, oval to polygonal, sieve cells; vessels; and dark-coloured substances (Fig. 1i).

Physical parameters

All physiochemical parameters, including identification of ash content, acid-insoluble ash content, ethanol-soluble extract content, moisture content, water-soluble extract content, and pH value were assessed and found to be within permissible limits as specified in the Ayurvedic Pharmacopeia of India. The results are presented in Table 2.

Moisture content is a critical indicator of the quality of plant material; excessive or deficient moisture content of a substance can adversely impact its physical properties. An excess of water in herbal materials will encourage microbial growth, whereas inadequate moisture content will lead to deterioration in the bioactivity and quality of the plant material²⁹. As a rule, the moisture content should not be more than 50% of the total soluble content of the plant material³⁰. All the tested herbal powders, claimed as *Kakoli*, fulfilled this fundamental principle, and exhibited moderate-to-low moisture content.

Additionally, ash content is a measure of the concentration of minerals and other inorganic matter present in plant material. Total ash content alone, however, is not sufficient in determining the quality of plant materials; therefore, acid-insoluble and water-soluble ash content are both used as indices of the quality of herbals^{31,32}. All the tested herbal materials exhibited a total ash content below the permissible limit of 6%, in line with the required API specification.

Furthermore, water-soluble extractive value plays an important role in the evaluation of herbal samples, where a lower extractive value indicates adulteration or incorrect processing of the herbals^{33,34}. In the present study, all the tested herbal powders exhibited moderate-to-high water-soluble extractive value, thereby indicating their purity. The highest water-soluble extractive value was found in *R. purpurea*

Table 2 — Physiochemical parameters of powdered herbal materials proposed as *Kakoli*

Parameter	Moisture content	Total ash	Acid insoluble ash	Water soluble ash	Alcohol soluble extractive	Water soluble extractive	pH
<i>Roscoea purpurea</i> Sm.	3.74%	4.56%	0.66%	1.88%	5.91%	10.05%	6.50
<i>Roscoea capitata</i> Sm.	3.39%	5.16%	0.65%	1.89%	6.59%	8.66%	6.42
<i>Roscoea alpina</i> Royle	3.79%	4.63%	0.69%	1.76%	6.23%	8.70%	6.53

Sm. (~ 10.05%), followed by *R. alpina* Royle (~ 8.70%) and *R. capitata* Sm. (~ 8.66%).

The pH values of all the tested powders were found to be less than 7 and more than 6, thereby indicating all 3 powders to be safe for human use³⁵.

Phytochemical analysis

Total polyphenols, flavonoids, saponin, and proanthocyanidin content were determined spectrophotometrically, as per the methods described for the present study. Total polyphenol content in the tested herbal powders were assessed using gallic acid as the standard ($y = 0.0135x + 0.8363$; $R^2 = 0.9907$). The powdered material of *R. capitata* Sm. contained the highest concentration of polyphenols (~ 111.31 mg/g eq. gallic acid), followed by *R. alpina* Royle (~ 68.2 mg/g eq. gallic acid) and *R. purpurea* Sm. (~ 44.57 mg/g eq. gallic acid), as shown in Table 3a. Polyphenols are known to contain one or more phenolic rings, thereby having abundant hydroxyl functional groups, which serve as favourable sources of hydrogen donors to free radical moieties and, therefore, are antioxidants of choice. Our study established that all the *Roscoea* species investigated contained considerable concentrations of polyphenols,

with associated antioxidant activity, which might ultimately be responsible for their health-invigorating and rejuvenating potential³⁶.

Total flavonoid content in the tested herbal powders was assessed using quercetin as the standard ($y = 0.0136x - 0.1348$; $R^2 = 0.9817$). The results showed that the powdered material of *R. alpina* Royle and *R. purpurea* Sm. contained moderate-to-high concentrations of flavonoids (*R. alpina* Royle ~ 28.58 mg/g eq. quercetin & *R. purpurea* Sm. 28.14 mg/g eq. quercetin), as shown in Table 3b. Flavonoids are often considered to be indicators of the antioxidant activity of a species³⁷. The present study shows that *R. alpina* Royle and *R. purpurea* Sm. might be equated with *Kakoli*, as both species contain ample quantities of flavonoids, making them effective as a tonic or rejuvenator, similar to *Kakoli*⁸.

Total saponin content in the tested herbal powders was assessed using diosgenin as the standard ($y = 0.0047x + 0.026$; $R^2 = 0.9955$). The results showed that the powdered material of *R. purpurea* Sm. contained the highest concentration of saponins (~ 51.91 mg/g eq. diosgenin; 39.36 mg/g eq. *R. capitata* Sm. & 49.78 mg/g eq. *R. alpina* Royle), as shown in Table 3c.

Table 3a — Polyphenol content of powdered herbal materials proposed as *Kakoli*

Plant material	Value of Y = Absorbance ($y = 0.0135x + 0.8363$; $R^2 = 0.9907$)	Result mg/g eq. Gallic acid
<i>Roscoea purpurea</i> Sm.	1.438	44.57
<i>Roscoea capitata</i> Sm.	2.339	111.31
<i>Roscoea alpina</i> Royle	1.757	68.2

Table 3b — Flavonoid content of powdered herbal materials proposed as *Kakoli*

Plant material	Value of Y = Absorbance ($y = 0.0136x - 0.1348$; $R^2 = 0.9817$)	Result mg/g eq. Quercetin
<i>Roscoea purpurea</i> Sm.	0.248	28.14
<i>Roscoea capitata</i> Sm.	0.215	25.72
<i>Roscoea alpina</i> Royle	0.254	28.58

Table 3c — Saponin content of powdered herbal materials proposed as *Kakoli*

Plant material	Value of Y = Absorbance ($y = 0.0047x + 0.026$; $R^2 = 0.9955$)	Result mg/g eq. Diosgenin
<i>Roscoea purpurea</i> Sm.	0.272	51.91
<i>Roscoea capitata</i> Sm.	0.213	39.36
<i>Roscoea alpina</i> Royle	0.262	49.78

Table 3d — Proanthocyanidin content of powdered herbal materials proposed as *Kakoli*

Plant material	Value of Y = Absorbance ($y = 0.0014x + 0.179$; $R^2 = 0.9943$)	Result mg/g eq. Catechin
<i>Roscoea purpurea</i> Sm.	-	Below detection range
<i>Roscoea capitata</i> Sm.	0.199	14.28
<i>Roscoea alpina</i> Royle	-	Below detection range

Saponins are known for their spermatogenic activity, aiding the activation of gonadal tissues and the central nervous system by means of an NO-dependent mechanism³⁸. *Roscoea purpurea* Sm. might, therefore, show similar spermatogenic activity to *Kakoli*, based on the presence of saponins⁹.

The total anthocyanidin concentration of the tested herbal powders was found to be below the quantifiable range; however, proanthocyanidin was detectable in *R. capitata* Sm. (~ 14.28 mg/g eq. catechin). The other two herbal powders, namely, *R. purpurea* Sm. and *R. alpina* Royle, either did not contain proanthocyanidin, or the concentration was below the range of detection, as shown in Table 3d. Proanthocyanidins are reportedly a promising source of therapeutic moieties, which can avert abnormal reproductive outcomes and hence provide symptomatic relief of reproductive ailments³⁹. *Roscoea capitata* might exhibit aphrodisiac activity due to the presence of proanthocyanidins, similar to the natural sexual rejuvenator, *Kakoli*⁹.

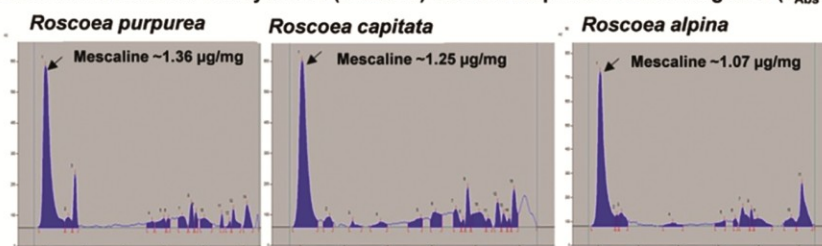
Chromatographic & spectroscopic analysis

The HPTLC analysis showed several constituents with substantial, distinct peaks in R_f range: 0.07–0.99

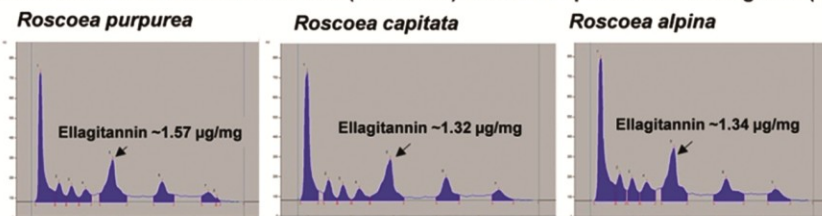
in all the herbal powder samples analysed (Fig. 2). The quantification of mescaline, ellagitannin, and gallic acid in the herbal samples was authenticated by a parallel run of standard marker compounds, followed by visualisation under a TLC scanner at $\lambda_{Abs}=254nm/\lambda_{Excitation} = 366 nm$. The investigation, comprising diverse concentrations of mescaline, ellagitannin, and gallic acid as standard marker compounds, showed that *R. purpurea* Sm. contained the highest concentrations of mescaline (~ 1.36 $\mu\text{g}/\text{mg}$) and ellagitannin (~ 1.57 $\mu\text{g}/\text{mg}$), as shown in Table 4. Mescaline might be responsible for attributing sedative and tonic properties to *R. purpurea* Sm.⁴⁰, while ellagitannin confers *R. purpurea* Sm. with probable antioxidant and rejuvenating properties⁴¹.

The LC-MS fingerprint analysis of the powders of *R. purpurea* Sm., *R. capitata* Sm., and *R. alpina* Royle revealed the presence of gallic acid, catechin, caffeic acid, and quercetin at retention times (RTs) of 7.314, 13.902, 14.982, and 20.362 min ($\lambda = 270 nm$), respectively, as shown in Fig. 3. The eluted samples were subjected to mass spectral analysis, and the fragmentation patterns of major fragment ions were

Mobile Phase 1: Chloroform: Acetone: Diethylamine (70:20:10) with the respective Chromatograms ($\lambda_{Abs} = 254 nm / \lambda_{Abs} = 366 nm$)



Mobile Phase 2: Toluene: Acetone: Glacial acetic acid (100:3:0.07) with the respective Chromatograms ($\lambda_{Abs} = 254 nm / \lambda_{Abs} = 366 nm$)



Mobile Phase 3: Benzene: Ethyl acetate: Glacial acetic acid (90:5:5) with the respective Chromatograms ($\lambda_{Abs} = 254 nm / \lambda_{Abs} = 366 nm$)

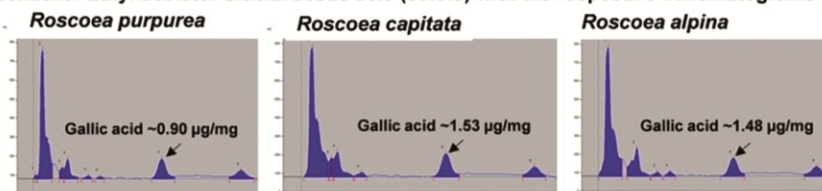


Fig. 2 — HPTLC of Herbal powder of *Roscoea* species. Mescaline, ellagitannin and gallic acid was observed in *Roscoea purpurea* Sm., *Roscoea capitata* Sm. and *Roscoea alpina* Royle with maximum concentration of mescaline, ellagitannin and gallic acid in *Roscoea purpurea* Sm. as 1.36, 1.57 and 0.90 $\mu\text{g}/\text{mg}$, respectively

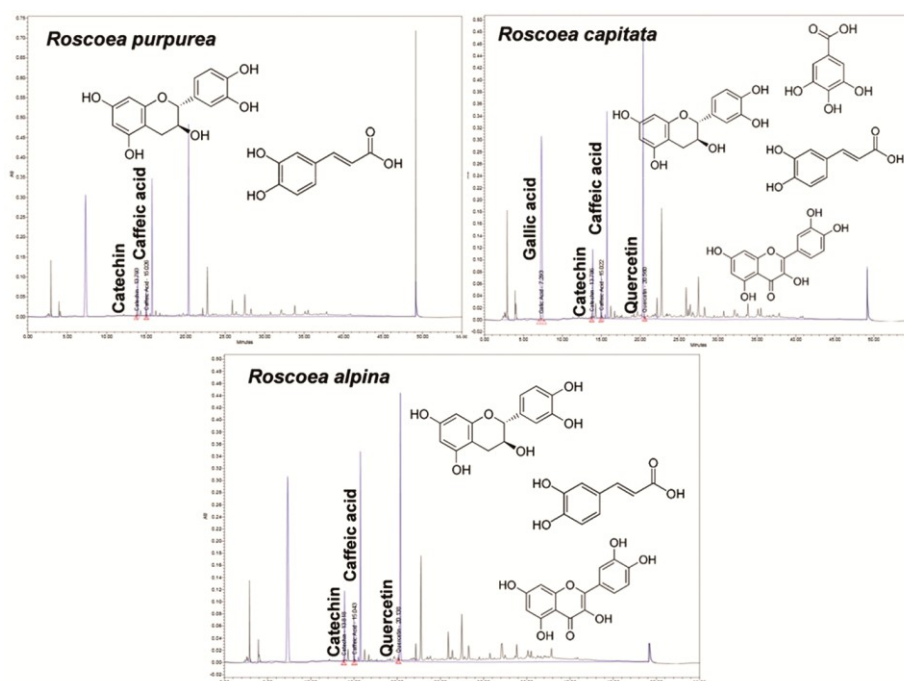


Fig. 3 — LCMS of Herbal powder of *Roscoeae* species. Gallic acid, catechin, caffeic acid and quercetin was observed in *Roscoeae purpurea* Sm., *Roscoeae capitata* Sm. and *Roscoeae alpina* Royle, wherein catechin and caffeic acid was found in all the tested herbal samples. Moreover, *Roscoeae capitata* Sm. showed the presence of all the standard phytochemicals

Table 4 — HPTLC based Quantitation of Standard Markers in powdered herbal materials proposed as *Kakoli*

Mobile Phase	Quantity of Standard Marker Compound		
	<i>Roscoeae purpurea</i> Sm.	<i>Roscoeae capitata</i> Sm.	<i>Roscoeae alpina</i> Royle
Chloroform: Acetone: Diethyl amine (70:20:10) (Quantitation of Mescaline)	1.36 µg/mg of dried powder	1.25 µg/mg of dried powder	1.07 µg/mg of dried powder
Toluene: Acetone: Glacial acetic acid (100:3:0.07) (Quantitation of Ellagitannin)	1.57 µg/mg of dried powder	1.32 µg/mg of dried powder	1.34 µg/mg of dried powder
Benzene: Ethyl acetate: Glacial acetic acid (90:5:5) (Quantitation of Gallic acid)	0.90 µg/mg of dried powder	1.53 µg/mg of dried powder	1.48 µg/mg of dried powder

Table 5 — HPLC based Quantification of marker compounds present in three species of *Roscoeae* proposed as *Kakoli* ($\lambda = 270$ nm)

Marker Compound	<i>Roscoeae purpurea</i> Sm.	<i>Roscoeae capitata</i> Sm.	<i>Roscoeae alpina</i> Royle
Gallic Acid	Below detectable range	0.00077% w/w	Below detectable range
Catechin	0.00063% w/w	0.00123% w/w	0.000577% w/w
Caffeic Acid	0.00152% w/w	0.00308% w/w	0.00218% w/w
Quercetin	Below detectable range	0.000047% w/w	0.00029% w/w

recorded. The fragmentation pattern, as revealed in the full ion spray mass spectrum, showed varying concentrations of these markers in the tested herbal samples (Table 5). Catechin and caffeic acid were found in all the tested herbal powders, with the highest concentrations being found in *R. capitata* Sm. (catechin ~ 0.00123% & caffeic acid ~ 0.00308%). The most probable bioactivity linked to these phenolic compounds could be their potent antioxidant behaviour, which, in turn, endows them with

rejuvenating, anti-aging, and vitality-providing capabilities^{9,25,42}. The present study, therefore, supports the comparison of *Roscoeae* species with the renowned medicinal herb of the *Astavarga* group, *Kakoli*.

Correlation analysis

A comparative table was drawn up, based on literary as well as experimental analyses, to identify the most closely allied herbal species of *Roscoeae*, which can be equated to *Kakoli* (Table 6). A similarity score was also given for each parameter,

Table 6 — Correlation Analysis of *Roscoea* species with *Kakoli* as mentioned in ancient scriptures

Literary Resource based Comparison						
Parameter of Comparison	<i>Kakoli</i>	<i>Roscoea purpurea</i> Sm.	<i>Roscoea capitata</i> Sm.	<i>Roscoea alpina</i> Royle	Similarity Score with <i>Kakoli</i>	Inference
Habitat	Alpine grassland, steppes, grassy hillsides, damp gullies and stony slopes	Alpine grassland, forest edges and on moist rock faces	Open grassland, damp gully	Conifer forests, damp grass, on shady banks or slopes, in gorge beds	<i>Roscoea purpurea</i> Sm. – 0.4 <i>Roscoea capitata</i> Sm. – 0.2 <i>Roscoea alpina</i> Royle – 0.2	<i>Roscoea purpurea</i> Sm. is proposed as <i>Kakoli</i> .
Distribution	Himalayan region of Pakistan, Bhutan, Tibet; Central & Eastern Himalayan region from Uttarakhand to Sikkim and Assam; 1500-3300 m	Himachal Pradesh (Shimla), Uttarakhand (Mussoorie), Garhwal, Assam, Nepal, Bhutan; 2440-2740 m	Northwest Nepal (Kathmandu); 1220-2600 m	Sikkim, East Himalaya, West Himalaya, Bhutan, Nepal, Pakistan, Tibet; 2130-4270 m	<i>Roscoea purpurea</i> Sm. – 0.57 <i>Roscoea capitata</i> Sm. - 0 <i>Roscoea alpina</i> Royle– 0.57	<i>Roscoea purpurea</i> Sm. and <i>Roscoea alpina</i> Royle are proposed as <i>Kakoli</i> .
Distinguishing Feature	Purplish rhizome exuding milky latex; purple, pale lilac or white flowers	Inflorescence borne on peduncle hidden by leaf sheaths; capsules produced within leaf sheaths; calyx glabrous; epigynous glands 7.5-9.5 mm long; light purple or pale lilac flowers	Inflorescence borne on peduncle exerted from leaves; capsules exposed; calyx pubescent; epigynous glands about 5 mm long; light pink or white flowers	Staminodes circular; bracts very short, under 1-5 cm long; deep purple flowers	<i>Roscoea purpurea</i> Sm. – 0.67 <i>Roscoea capitata</i> Sm.– 0.33 <i>Roscoea alpina</i> Royle - 0	<i>Roscoea purpurea</i> Sm. is proposed as <i>Kakoli</i> .
Leaf Morphology	5-6 in number, lanceolate, 15 cm x 1.2-2.5 cm	4-8 in number, elliptic, lanceolate to oblong-ovate, 14-20 cm x 1.5 – 4.7 cm	3-7 in number, linear, rarely lanceolate, 9-16 cm x 1.2-1.8 cm	1-4 in number, mostly linear, broadly elliptic or lanceolate, 2.5-17 cm x 1.3 – 3.5 cm	<i>Roscoea purpurea</i> Sm. - 1 <i>Roscoea capitata</i> Sm.– 0.67 <i>Roscoea alpina</i> Royle – 0.67	<i>Roscoea purpurea</i> Sm. is proposed as <i>Kakoli</i> .
Flower Morphology	Calyx green; corolla purple, pale lilac or white	Calyx pale green; corolla light purple, pale lilac, mauve, pink, red or white	Calyx green; corolla light pink or white	Calyx green, spotted; corolla deep purple or lilac	<i>Roscoea purpurea</i> Sm. – 0.67 <i>Roscoea capitata</i> Sm. – 0.33 <i>Roscoea alpina</i> Royle – 0.33	<i>Roscoea purpurea</i> Sm. is proposed as <i>Kakoli</i> .
Therapeutic Claims	Anti-anaemic, Anti-diarrhoeal, Antidote, Anti-haemorrhagic, Anti-malarial, Antipyretic, Anti-rheumatic, Anti-tubercular, Anti-tussive, Aphrodisiac, Tonic	Anti-malarial, Tonic, Anti-rheumatic, Aphrodisiac	Aphrodisiac	Aphrodisiac	<i>Roscoea purpurea</i> Sm. – 0.36 <i>Roscoea capitata</i> Sm. – 0.09 <i>Roscoea alpina</i> Royle– 0.09	<i>Roscoea purpurea</i> Sm. is proposed as <i>Kakoli</i> .
Experimental Resource based Comparison						
Parameter of Comparison		<i>Roscoea purpurea</i> Sm.	<i>Roscoea capitata</i> Sm.	<i>Roscoea alpina</i> Royle	Discussion	
pH		6.50	6.42	6.53	Suitability for human use.	
Moisture content		3.74%	3.39%	3.79%	Indistinguishable differences in moisture content; Within permissible limits.	

(contd.)

Table 6 — Correlation Analysis of *Roscoea* species with *Kakoli* as mentioned in ancient scriptures (*contd.*)

Parameter of Comparison	Literary Resource based Comparison			Discussion
	<i>Roscoea purpurea</i> Sm.	<i>Roscoea capitata</i> Sm.	<i>Roscoea alpina</i> Royle	
Ash content	4.56%	5.16%	4.63%	Indistinguishable differences in moisture content; Within permissible limits.
Acid insoluble ash content	0.66%	0.65%	0.69%	
Water-soluble extract content	10.05%	8.66%	8.70%	Highest Water-soluble extract content in <i>Roscoea purpurea</i> Sm., indicating it to be free from adulteration.
Ethanol-soluble extract content	5.91%	6.59%	6.23%	Highest Ethanol-soluble extract content in <i>Roscoea purpurea</i> Sm., indicating it to be free from adulteration.
Polyphenol content	44.57 mg/g eq. Gallic acid	111.31 mg/g eq. Gallic acid	68.2 mg/g eq. Gallic acid	Highest concentration of polyphenols in <i>Roscoea capitata</i> Sm., indicating its significant antioxidant nature, thereby endowing rejuvenation properties to the plant species.
Flavonoid content	28.14 mg/g eq. Quercetin	25.72 mg/g eq. Quercetin	28.58 mg/g eq. Quercetin	Moderate-to-high concentration of flavonoids in <i>Roscoea purpurea</i> Sm. and <i>Roscoea alpina</i> Royle, indicating their significant antioxidant nature and rejuvenation properties.
Saponin content	51.91 mg/g eq. Diosgenin	39.36 mg/g eq. Diosgenin	49.78 mg/g eq. Diosgenin	Highest concentration of saponin in <i>Roscoea purpurea</i> Sm., indicating its action to relax blood vessels/ corpus cavernosum (thus, for the treatment of men suffering from erectile dysfunction). Moreover, saponins have also been shown to improve libido, sexual activity, and intracavernous pressure.
Proanthocyanidin content	Not reported	14.28 mg/g eq. Catechin	Not reported	Highest concentration of proanthocyanidin in <i>Roscoea capitata</i> Sm., indicating its protective role during the cell proliferation changes in germinal cells.

deduced by dividing the parameters present in each *Roscoea* species by all the traits mentioned for *Kakoli* in ancient scriptures. Based on the presence and absence of comparison parameters in each proposed species, firstly a binary score was given for each species, wherein '0' meant absence and '1' means the presence of a given parameter. Secondly, the similarity score for each parameter, relating to each proposed species was deduced by using the following formula;

$$\text{Similarity Score} = (S - \text{Min } S) / (\text{Max } S - \text{Min } S), \text{ eqn. 1}$$

Wherein S refers to the binary score of given species for a particular parameter; Min S is the minimum score among the subset for a particular parameter; & Max S is the maximum score among the subset for a particular parameter.

The comparison with the literary resource-based parameters showed that the highest total score of similarity was obtained for *R. purpurea* Sm. (~ 3.67),

followed by *R. alpina* Royle (1.86) and *R. capitata* Sm. (~ 1.62). The experimental resource-based comparison of the present study also provided adequate authentication for establishing the botanical identity of *Kakoli* to be *R. purpurea* Sm.²⁸.

Conclusion

The major contradiction associated with traditional medicines is the confusion caused by using a single common name for more than one botanically distinct but similar species. *Kakoli* is an example of such controversial drug, which has been described both in ancient scriptures and in modern research-based literature. In the present study, the correlation analyses based on various physical, pharmacognostic, phytochemical, spectroscopic, and chromatographic parameters of identification, as well as differentiation among different *Roscoea* species, suggest that *R. purpurea* Sm. might be the closest and most probable source of *Kakoli*.

Supplementary Data

Supplementary data associated with this article is available in the electronic form at [https://nopr.niscpr.res.in/jinfo/ijtk/IJTK_23\(08\)\(2024\)747-759_SupplData.pdf](https://nopr.niscpr.res.in/jinfo/ijtk/IJTK_23(08)(2024)747-759_SupplData.pdf)

Acknowledgements

The authors are thankful to Swami Ramdev Ji for providing research amenities and support. We acknowledge Patanjali Research Foundation Trust for providing financial and scientific support required for the study. We also acknowledge the members of Patanjali research group who helped us in data collection.

Conflict of Interests

All the authors proclaim no conflict of interest in publication of this manuscript.

Author Contributions

The present work has been conceptualized by AB and designed by PT, SS, SN and RM. AV critically evaluated the final draft.

Data Availability

The data used is available at Mendeley Data, V1, doi: 10.17632/vxbk57t6nx.1.

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