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New insights into the morphology of *Malaxis acuminata* D Don

YASH PAL SHARMA, JADHAV RANI, RAVINDER RAINA and KUMARI BANDANA

Department of Forest Products
Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan 173230 HP
Email for correspondence: yashuhf@gmail.com

ABSTRACT

Correct botanical identity of important ayurvedic drugs is generally a matter of confusion as most of these are known by their local/trade names. This usually hampers dedicated conservation and domestication efforts as several of high in demand Ayurvedic herbs suffer due to unsustainable harvests. The situation is grimmer in such herbs which have widespread usage and demand. *Malaxis acuminata* D Don syn *Microstylis wallichii* Lindl is an important ingredient of an important polyherbal Ayurvedic formulation 'Ashtavarga'. This species is variously known as Jivakah (as per the Ayurvedic Pharmacopoeia of India), Jivya, Dirghayu, Cirajivi (in Sanskrit), Jeevak (in Hindi and English), Jeevakam (in Malayalam and Tamil), Jeevakamu (in Telugu). These different names (for the same identity) generally lead to misidentification, unintentional adulteration etc with an adverse impact on its potency. The present studies have brought out several hitherto unknown morphological features like palisade ratio (0.84 ± 0.03), stomatal index (8.43 ± 0.80), stomata number (70.34 ± 2.28), stomata size (0.036 ± 0.0005mm ×0.03 ± 0.0006 mm) and type of stomata (anomocytic) of *M acuminata* that can be helpful in its correct identification. Visual characterization of the economic part of this species has also been attempted to differentiate between almost globular pseudobulb and rhizome.

Keywords: *Malaxis acuminate*; modified stem; rhizome; pseudobulb

INTRODUCTION

Malaxis acuminata D Don syn *Microstylis wallichii* Lindl (Orchidaceae) is an important ingredient of polyherbal Ayurvedic formulation 'Astavarga' and is variously known as Jivakah (as per the Ayurvedic Pharmacopoeia of India), Jivya, Dirghayu, Cirajivi (in Sanskrit), Jeevak (in Hindi and English), Jeevakam (in Malayalam and Tamil) and Jeevakamu in Telugu (Anon 2006). It is a terrestrial, perennial and endangered medicinal orchid of Himalayan region (Shukla and Chaubey 2008. Clarke 1885) being used in Ayurveda for the preparation of

Chayawanprash, Astavarga Churna, Chitrakadi Taila, Vachadi Taila, Vajikarn Ghrita etc (Anonymous 2006, Balkrishna et al 2012, Dhyani et al 2010). The species is used to cure tuberculosis and is a great aphrodisiac (Chauhan 1990). Its pseudobulbs are sweet, refrigerant, aphrodisiac, styptic, antidysenteric, febrifuge, tonic and useful in the conditions of sterility, seminal weakness, internal and external hemorrhages, dysentery, fever, emaciation, burning sensation as well as general debility (Balkrishna et al 2012).

M acuminata is mostly distributed in India in temperate to subtropical Himalayas (Clarke 1885) at an altitude of 1200-2100 m from Himachal Pradesh, Uttarakhand to Arunachal Pradesh, Assam, Nagaland, Manipur, Mizoram and Tripura and at an altitude of 1500-1800 m in Khashi hills (Balkrishna et al 2012).

Due to high demand by the Ayurvedic industries the species has been and is being massively overexploited from its natural distribution regions without any attempt to cultivate. Due to unscientific harvesting, overexploitation and habitat destruction the existence of *M acuminata* has been threatened and it has been notified in CITES Appendix-II for ensuring its conservation (Lange and Schippmann 1999). Due to its widespread demand and limited availability it is being extremely adulterated by rootstock of *Ipomoea digitata* (Chinmay et al 2011) and is also being substituted with Ashwagandha (*Withania somnifera*), Safed Musli (*Chlorophytum borivilianum*) and *Lillium wallichianum* (Balkrishna et al 2012).

The morphological features of *Malaxis acuminata* D Don syn *Microstylis wallichii* Lindl have been described by various workers (Clarke 1885, Collett 1921, Polunin and Stainton 1977, Bose et al 1980, Chowdhery and Wadhwa 1984, Balkrishna et al 2012, Yonzone et al 2013) but information on its important pharmacogonostic features like palisade ratio, stomatal index, stomata number, stomata size and type of stomata is still lacking and economic part of the plant (swollen stem) has been described differently. The present study was initiated to generate useful information on its morphology that would ultimately lead to correct identification of drug Jivakah (*M acuminata*).

MATERIAL and METHODS

The present study was carried out at Medicinal and Aromatic Plants Research Farm, Shilly (altitude 1550 m amsl, latitude- N 30^0 54' 30" and longitude E 77^0 07' 30") of Dr YS Parmar University of Horticulture and Forestry, Solan, HP during 2011-2013. Propagules of *Malaxis acuminata* were collected from two distinctly located populations viz Shilly wildlife sanctuary (altitude 1550 m amsl) and Churdhar

wildlife sanctuary (altitude 2850 m amsl). The plants of both the populations were raised from propagules under uniform conditions in field at spacing of 15 x 10 cm. Qualitative and quantitative morphological features were recorded on randomly selected 25 plants of each population as per Lawrence (1951), Collett (1921), Kaufman et al (1989) and Weberling (1989) for two growing cycles from sprouting of propagules till senescence.

The palisade ratio was calculated on single epidermal cell basis and was based on 10 observations from different leaves. The leaves were boiled in chloral hydrate solution till they became completely dechlorophylled. Number of palisade cells below four adjacent epidermal cells in dechlorophylled leaves was counted. Stomatal studies were made from freshly peeled lower epidermis layer. Fresh peels were mounted in saffranine (2% aqueous) covered with cover slips and observed under microscope. Stomatal index is the percentage which the number of stomata form to the total number of epidermal cells including stomata each stomata being counted as one cell. Stomatal index was calculated by formula:

$$I = (S \times 100)/(E+S)$$

where I= Stomatal index, S= Number of stomata per unit area,

E = Number of epidermal cells in the same unit area

The type of stomata present was determined on the basis of stomatal classification as given by Fahn (1967). The size of the stomata was measured using ocular and stage micrometer (ERMA, Japan) and the number of stomata present per mm^2 area of lamina were referred as stomata number.

RESULTS

The qualitative as well as quantitative morphological features are consolidated in Table 1and 2 as described below:

Habit: Plants of *Malaxis acuminata* were erect, small with the stem composed of aerial flowering axis (which developed in flowering season only) and basal swollen stem resembling to rhizome like structure bearing nodes and internodes arising from the base of mother rhizome. Several adventitious roots arose below this rhizomatous structure firmly anchoring the plant to the soil. Initially mother rhizome was mostly upright remaining so before becoming horizontal and giving rise to two to four pseudobulbs and basal swollen stem gave one daughter rhizome (Plate1-7). These pseudobulbs once detached gave rise to new plants during next growth cycle.

Modified stem: The modified stem occured towards the base of the plant and was composed of rhizome like structure and pseudobulbs (growing on the surface of

Table 1. Qualitative morphological features of *Malaxis acuminate*

Parameter/ plant part	Feature
Habit	Erect, perennial
Stem	Erect, modified into rhizome like structure
Root	Adventitious arising from base of rhizome (fibrous)
Leaf	Simple, margins undulate, ovate-lanceolate, acute to acuminate, narrowed to sheathing base, petiolate
Inflorescence	Inflorescence racemose, peduncle ribbed
Bract	lanceolate, sub-acute, reflexed
Flower	Bracteates, zygomorphic, bisexual, complete, epigynous, greenish yellow tinged with purple spot
Calyx	Sepals 3, dorsal sepal linear-oblong, sub-acute, lateral sepals oblong, obtuse
Corolla	Petals 3, petals linear, obtuse, margins recurved, lip slightly convex, base with auricles, narrowly ovate-sagittate
Fruit	Capsule
Seed	Microscopic, powdery
Pseudobulb	Globular to conical
Rhizome	Elongated and tapering

mother rhizome) (Plate1-7). The rhizome was horn like structure, elongated and tapering with broader end towards base. The pseudobulbs were more or less globular to conical and produced 2-4 per mother rhizomes. Both rhizomes and pseudobulbs were covered by sheathing leaf bases which appeared on nodes marginally covering them. Both rhizome and psedobulbs were smooth, shining and green (without sheathing leaves) (Plate1-2, 4) when fresh turning to redish-brown upon drying (Plate1-8).

Leaf: In both the germplasms leaves were simple, ovate to lanceolate, petiolated and lamina entire with undulating margin having acute to acuminate tip with a sheathing (attenuate) base and parallel venation. The adaxial surface was dark green while abaxial was light green in colour.

Leaf constants: Leaf constants viz palisade ratio (0.84 ± 0.03), stomatal index (8.43 ± 0.80), stomata number (70.34 ± 2.28), stomata size (0.036 ± 0.0005 mm × 0.03 ± 0.0006 mm) and anomocytic type

Table 2. Quantitative morphological features of *Malaxis acuminata*

Parameters	Propagule	
	Pseudobulb	Rhizome
Plant height (cm)*	6.70 ± 0.24 (6-10)	10.45 ± 0.34 (9-13)
Leaf		
# leaves/plant	4.10 ± 0.12 (4-5)	4.15 ± 0.15 (4-5)
Leaf length (cm)	7.21 ± 0.14 (3-14)	11.51 ± 0.28 (3-21)
Leaf width (cm)	2.83 ± 0.14 (1-5)	4.95 ± 0.33 (1.2-7.5)
# costae/lamina	4.76 ± 0.04 (3-7)	5.74 ± 0.13 (3-7)
Root length (cm)	6.20 ± 0.30 (3-9)	7.84 ± 0.52 (4-14)
Rhizome		
Length (mm)	48.04 ± 1.76 (38-63)	66.51 ± 2.65 (44-84)
Breadth (mm)	12.38 ± 0.45 (9-13)	16.42 ± 0.8 (15-20)
Pseudobulb		
Length (mm)	12.94 ± 0.32 (9-20)	17.65 ± 0.67 (10-21)
Breadth (mm)	8.82 ± 1.8 (5-12)	11.16 ± 1.82 (6-14)

Values in parentheses are ranges, *At vegetative stage without flowering spike

of stomata (Plate 2-13) are being reported for the first time in this species.

Inflorescence and flower: During the first year of growth after planting inflorescences were produced by rhizomes propagules only and the pseudobulbs gave only vegetative growth. In both the germplasms flowers were born in terminal racemes. Each flower was subtended by a bract that was pubescent, lanceolate, sub-acute and greenish purple. The flowers were similar in both the germplasm being bracteate, zygomorphic, bisexual, complete and epigynous with a prominent purple coloured spot in the centre of labellum with the surrounding labellum as well as sepals, petals, bract and pedicel being yellow at the full bloom stage. The flower colour gradually changed later on which became entire purple including bract as well as pedicle (Plate 2-11). This colour change signalled senescence of flowers which may or may not develop fruits.

Calyx was composed of three sepals with one dorsal and two laterals. The dorsal sepal was marginally longer and

Table 3. Leaf constants

Constant	#/type
Palisade ratio	0.84 ± 0.03
Type of stomata	Anomocytic
Size of stomata	0.036 ± 0.0005mm × 0.03 ± 0.0006 mm
Stomatal index	8.43 ± 0.80
Stomata number	70.34 ± 2.28

Table 4. Quantitative floral parameters of *Malaxis acuminata*

Parameter	Value
Length of flowering axis (cm)	19-36 (24.44 ± 1.58)
# flowers/plant	8-35 (13.23 ± 2.00)
Bract	
Length (mm)	3-6 (3.8 ± 0.15)
Diameter (mm)	2
Flower size	
Length (cm)	1.2-1.5 (1.28 ± 0.03)
Spread (cm)	1 (1.05 ± 0.02)
Calyx	
# sepals	3
Lateral sepals size (mm)	6×3
Dorsal sepal size (mm)	7-8 × 2-3 (7.1 ± 0.1 × 2.2 ± 0.13)
Corolla	
# petals	3
Lateral petals size (mm)	6 × 1
Lip size (mm)	8-9 × 6 (8.2 ± 0.13 × 6)
Gynostemium	
# pollinia	4
Size of pollinia(mm)	0.32-044 × 0.120.2 (0.40 ± 0.006 × 0.16 ± 0.004)
Size of ovary (mm)	3 × 1
# carpels	3
Size of capsule (mm)	18.59-25.61 × 4.2-4.7 (21.31 ± 0.83 × 4.72 ± 0.15)
Size of seed (mm)	0.27-0.54 × 0.09-0.21 (0.45 ± 0.02 × 0.14 ± 0.01)

Values in parentheses are mean values

narrower as compared to lateral sepals. Both dorsal and lateral sepals were oblong shaped with the former being slightly more linear. However sub-acute tip in dorsal and obtuse tip in lateral sepals were observed. Corolla was composed of three petals (alternating with three sepals) of which one was ventral and two laterals per flower. The ventral petal was modified into a shield shaped large labellum or lip which formed the prominent part of the flower. The gynostemium or column situated in the

centre of the flower formed by the fusion of stamens and stigma represented the reproductive part of its flower. The anther was represented by an anther cap bearing four pollinia. The pollinia were arranged in two groups and each group contained two pollinia (Plate 1-10). Just below the anther on the ventral surface of the column there was a hollow cavity representing sticky and viscid stigma. Ovary was epigynous, tricarpellary, syncarpous and unilocular with parietal placentation containing numerous minute ovules (Plate 2-14).

Fruit and seed: Single cylindrical capsule was produced per flower. Fruits were greenish coloured when immature and turned pale brownish on maturity (Plate 2-15). Seeds were numerous, extremely minute and powdery (Plate 2-16). Weight of seeds per capsule was 13.17 ± 0.25 mg.

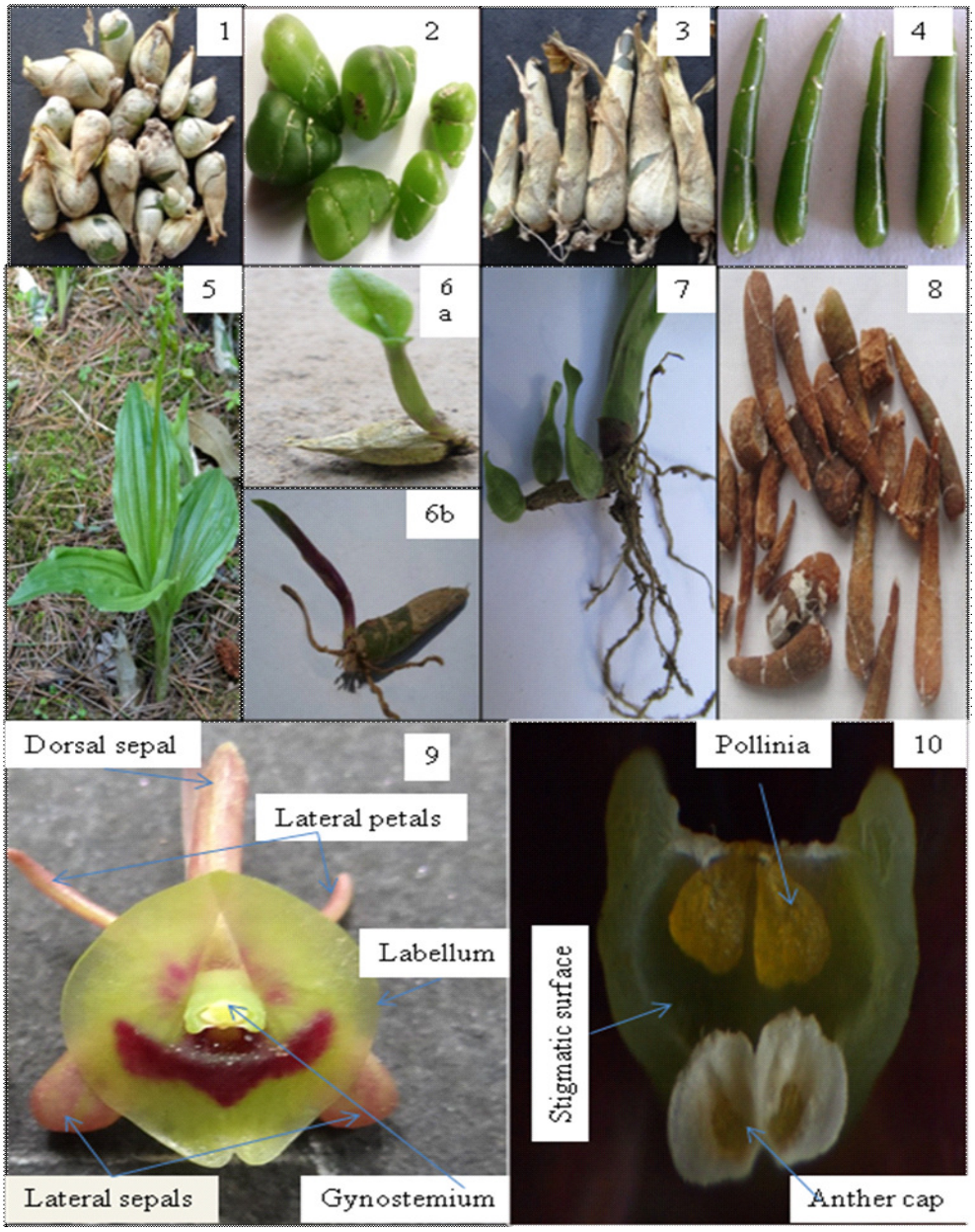
DISCUSSION

Correct botanical identity of important Ayurvedic drugs is generally a matter of confusion as most of these are known by their local/trade names. A perusal of literature reveals that *Malaxis acuminata* is very well known as far as its medicinal importance is concerned but information regarding its detailed morphological features is sketchy. Large information gaps are found in literature which impedes its successful conservation and domestication. Botanically the species has been variously known as *Crepidium acuminatum* (D Don) Szlach (Balkrishna et al 2012), *Microstylis wallichii* Lindl (Balkrishna et al 2012, Anon 2006) and *M acuminata* D Don (Balkrishna et al 2012, Anon 2006). However as per Bennet (1987) the accepted botanical name of the species is *Malaxis acuminata* D Don and has also been followed in present study.

The present studies were initiated to generate useful information that shall ultimately lead to correct identification of plants used as drug Jivakah. The general features of its plants like erectness, perennial habit, presence of modified stem at its base, leaf and floral details perfectly matched with the earlier reports (Collett 1921, Clarke 1985, Polunin and Stainton 1977, Yonzone et al 2013, Balkrishna et al 2012) .

However as per literature there emerged a slight doubt as far as the nature of modified stem was concerned. In literature the common term used is pseudobulb with conical more or less spherical shape. However on closer examination it was apparent that the modified stem present at its base presented two types viz (i) horn like structure that is elongated and tapering bearing adventitious roots (Plate 1-3, 4) at base (considered as rhizomes in the present study) and aerial flowering shoot at the top and (ii) smaller more or less globular to conical bulb shaped outgrowths (considered as pseudobulb in the present study) arising from mother rhizome (Plate 1-1, 2, 7).

Plate 1. New insights into the morphology of *Malaxis acuminata* D Don



1. Pseudobulbs with sheath 2. Pseudobulbs without sheath 3. Rhizomes with sheath 4. Rhizomes without sheath 5. Flowering plant 6a. Sprouting of pseudobulb 6b. Sprouting of rhizome 7. Pseudobulbs on mother rhizome 8. Dried rhizomes without sheath 9. Flower 10. Microscopic image of top of gynostemium