

## Comparative Biochemical and Phytochemical Analysis of Three Medicinal Terrestrial Orchid Species from Western Ghats, India

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### Abstract:

This study investigated the biochemical and phytochemical compositions of three medicinal terrestrial orchid species, *Calanthe sylvatica*, *Malaxis rheedii*, and *Satyrium nepalense*, collected from Chikmagalur, Karnataka, India. The analysis focused on the quantification of total sugars, reducing sugars, soluble proteins, amino acids, total phenols, flavonoids, and alkaloids. The results revealed that *S. nepalense* exhibited the highest concentrations of total soluble sugars ( $144.2 \pm 3.8 \mu\text{g/gDW}$ ), reducing sugars ( $186.5 \pm 6.2 \mu\text{g/gDW}$ ), soluble proteins ( $54.1 \pm 3.4 \mu\text{g/gDW}$ ), amino acids ( $98.6 \pm 3.5 \mu\text{g/gDW}$ ), total phenols ( $29.6 \pm 3.3 \text{ mg GAE/gDW}$ ), and alkaloids ( $37.1 \pm 2.4 \text{ mg AE/g dry weight}$ ), indicating its superior biochemical profile. *C. sylvatica* and *M. rheedii* also demonstrated significant phytochemical contents, with *C. sylvatica* having the highest flavonoid content ( $2.43 \pm 0.15 \text{ mg QE/g dry weight}$ ). These findings suggest that these orchid species possess considerable medicinal potential due to their rich biochemical and phytochemical compositions, supporting their traditional use in herbal medicine.

**Keywords:** *Calanthe sylvatica*, Flavanoids, *Malaxis rheedii*, Phenol and *Satyrium nepalense*

### I. INTRODUCTION

*Calanthe sylvatica* (Thouars.) Lindl. (Figure 1 A, B) is a terrestrial species features pseudobulbs and annulate stems. The leaves are numerous, tapering to a sheath with a ribbed petiole. The flowers, ranging from bright white to pale purplish pink, appear on long racemes. The bracts are persistent, with the side lobe of the lip varying in size and shape, and the spur may be inflexed, deflexed, or straight. It is found in the subtropical Himalayan ranges, Malaysia, Borneo, and South India. In the Western Ghats, it is widespread at higher elevations under shady floors of wet evergreen forests in regions such as Kodagu, Kudremukh, and Uttara Kannada; Ponnudi, Boacad, Kothyar, Naterickal, Mahendra Giri, and Kodaikanal. The flowering and fruiting period is from October to December. The flower juice is used to stop nosebleeds.<sup>1,2</sup>

*Malaxis rheedii* ex Sw. (Figure 1 C, D) is a terrestrial genus that may or may not have pseudobulbs, with terminal inflorescences bearing green doubly resupinate flowers. There are approximately 275 species distributed across tropical to temperate regions. In India, about 17 species are recorded, with six found in the Western Ghats. This terrestrial or lithophytic erect species has a stem that is sheathed and swollen at the base, and may be purplish or green. It has 3-5 membranous, ovate or lanceolate leaves, sheathed at the base. The inflorescence is a dense terminal raceme with maroon flowers. The bracts are lanceolate, the dorsal sepals linear, and the petals recurved or incurved. The capsules are oblong. It is widely distributed in western ghat areas specially in Tarave forests, Charmadi Ghats, Kotigehar, and along the way to Hebbe Falls (Kemmanundi), Dakshina Kannada. The flowering and fruiting period is from June to December. Their pseudobulbs are used as a tonic and form an ingredient of the important Ayurvedic medicine 'Astavarga,' which has therapeutic value.<sup>3,4</sup>

*Satyrium nepalense* (Figure 1 E, F) is a terrestrial, tuberous herb that thrives on grassy hilltops during the post-monsoon season. The plant features an inflorescence that is 25-40 cm long, forming a long spike with dense, fragrant pink flowers. The leaves are sessile, sheathing, and ovate-lanceolate. The flowers are non-resupinate, with oblanceolate bracts, oblanceolate dorsal sepals, and ovate-oblong lateral sepals. The petals are similar to the sepals, while the lip is cupular, crenulate, and posterior, with two long spurs attached on either side of the ovary. The column is arched, lacking a foot, and the pollinia are two, salverform, with distinct cordate viscidia. *Satyrium nepalense* is widely distributed across the Himalayas, Nepal, Burma, Sri Lanka, and South India (Karnataka, Kerala, and Tamil Nadu). Flowering and fruiting occur from October to January. The

underground tubers of *Satyrium nepalense* are sold in the market as salam-misri or salap. These tubers yield a useful tonic for curing malaria and dysentery.<sup>5,6</sup>

Although India is rich in orchid diversity, studies are scarce on them.<sup>7</sup> These medicinal orchid species have not yet been explored for their biochemical and phytochemical contents. Hence, the present study aimed to find and compare its nutrients and phytochemicals.

## II. MATERIAL AND METHODS

### 2.1 Collection of sample

Three medicinal terrestrial orchid species, *C. sylvatica*, *M. rheedii* and *S. nepalense* were collected from three different points of Chikmagalur, Karnataka, India. These points were Tiger Shola, Z-Point and Kemmangundi. They were identified by a taxonomist at the time of collection. Plant leaves were stored in a zip lock plastic cover and transported to the research lab.

### 2.2 Extraction of Sugars and Amino Acids

Oven-dried leaves (60°C) were homogenized in hot ethanol (80%) and centrifuged at 2000 rpm for 10 minutes, after which the supernatant was decanted. Subsequently, 3 ml of ethanol (80%) was added to the residue mixture, followed by another centrifugation. This extraction process was repeated twice to ensure the complete recovery of sugars and amino acids, with the remaining residue reserved for starch estimation. The supernatant was then cooled and evaporated to dryness on a petri dish in a boiling-water bath set at 45°C. The dried residue was eluted with 5 ml of 20% ethanol prior to analysis for sugars and amino acids.

### 2.3 Quantification of Total Sugars

The quantification of total sugars was conducted using the "anthrone method," as developed by Yemm and Willis in 1954. The concentration of total sugars was determined from a standard curve plotted with known concentrations of glucose.

### 2.4 Estimation of Reducing Sugars

The amount of reducing sugars was estimated using the "DNSA method" described by Sumner in 1935. The level of reducing sugars was calculated from a standard curve plotted with known concentrations of glucose.

### 2.5 Estimation of Free Amino Acids

The estimation of amino acid content was performed using the "Ninhydrin method" of Lee and Takahashi (1966). The concentration of free amino acids was determined from a standard curve plotted with known concentrations of glycine.

### 2.6 Preparation of Plant Extract for phytochemical analysis

The leaves were carefully separated from the stems and oven-dried for a week. The dried leaves were then finely powdered using an electric grinder. This powder was soaked in ethanol for three days, followed by filtration using Whatman No. 1 filter paper. The filtrates were then rotary-concentrated at 42°C to produce a dark brown semi-solid. Three times this extraction procedure was carried out. Following the dissolution of the dried extracts in 10% dimethyl sulfoxide (DMSO), a stock solution was made from which smaller quantities were made. The concentrated filtrate showed up as a greenish-brown paste that was semi-solid. To be used, 100 grams of the extract were dissolved in 50 milliliters of distilled water to create a stock solution.

### 2.7 Estimation of total phenol

To analyze total phenols, the Folin-Ciocalteu method is preferred for its simplicity, sensitivity, and reproducibility. The process begins by diluting the Folin-Ciocalteu reagent with distilled water at a 1:10 ratio. The plant material is then homogenized in a solvent like methanol or ethanol and filtered to remove solids. Extract aliquots are mixed with the diluted reagent and incubated at room temperature for 3 to 8 minutes. Sodium carbonate is added to neutralize the reaction and develop a blue color, indicative of phenolic content. The mixture is incubated in the dark for 30 minutes to 2 hours for full color development. The absorbance of the blue solution is measured at 760 nm using a spectrophotometer, and the total phenolic content is quantified against a standard calibration curve, typically using gallic acid. Results are expressed as milligrams of gallic acid equivalents (GAE) per gram of sample.

## 2.8 Quantification of Flavonoids

To estimate the flavonoid content in plant leaves, begin by collecting fresh leaves and thoroughly washing them with distilled water. Allow the leaves to dry at room temperature, then grind them into a fine powder using a mortar and pestle. For the extraction process, weigh 1 gram of the powdered leaf and mix it with 50 mL of 80% ethanol. Sonicate this mixture for 30 minutes at 30°C to enhance extraction efficiency. After sonication, centrifuge the mixture at 5000 rpm for 10 minutes and collect the supernatant, which contains the flavonoids. For quantification, prepare a calibration curve using quercetin as the standard, with concentrations ranging from 10 to 100 µg/mL. To measure the flavonoid content in the leaf extract, mix 1 mL of the extract with 1 mL of 2% aluminum chloride solution in ethanol. Incubate this mixture for 30 minutes at room temperature, allowing the flavonoids to complex with aluminum chloride, which enhances their absorbance. Measure the absorbance of the resulting solution at 415 nm using a UV-Vis spectrophotometer. Utilize the calibration curve to determine the flavonoid concentration in the extract, expressing the results as quercetin equivalents (mg QE/g dry weight) to quantify the flavonoid content accurately.

## 2.9 Quantification of alkaloids

To estimate the alkaloid content in plant leaves, start with the same sample preparation as for flavonoid estimation: wash the fresh leaves with distilled water, dry at room temperature, and grind into a fine powder. For extraction, weigh 1 gram of the leaf powder and mix it with 50 mL of 1% HCl in 70% ethanol. Heat this mixture under reflux for 2 hours, then cool and filter the extract, adjusting the pH to neutral with ammonium hydroxide. For quantification, create a calibration curve using atropine as the standard, with concentrations from 10 to 100 µg/mL. Mix 1 mL of the leaf extract with 5 mL of bromocresol green solution, then shake the mixture with 5 mL of chloroform for 30 minutes. Separate the chloroform layer and measure its absorbance at 470 nm using a UV-Vis spectrophotometer. Use the calibration curve to determine the alkaloid content, expressing it as atropine equivalents (mg AE/g dry weight).

## 2.10 Statistical analysis

All quantitative analysis was performed in triplicate. Mean and standard deviation were calculated for each phytochemical.

# III. RESULTS AND DISCUSSION

Tables 1 and 2 present the biochemical and phytochemical composition respectively. Which can be valuable for understanding their potential uses and benefits.

## 3.1 Total soluble sugar content

*S. nepalense* shows the highest concentration at 144.2±3.8µg/gDW, followed by *M. rheedii* at 140.8±5.0 µg/gDW, and *C. sylvatica* at 136.7±4.1 µg/gDW. The relatively higher sugar content in *S. nepalense* suggests it might be more effective in processes where higher sugar concentrations are advantageous, such as in the production of bioactive compounds or as a sweetener in food products.<sup>8</sup>

## 3.2 Reducing sugar

*S. nepalense* again leads with 186.5±6.2 µg/gDW, while *M. Maxalis rheedii* and *C. sylvatica* follow closely with 184.6 µg/gDW and 182.8 µg/gDW, respectively. Reducing sugars are important as they participate in various biochemical reactions, including the Maillard reaction, which is crucial in food science for flavor and color development during cooking. The slightly higher content in *S. nepalense* may imply it has a marginally better capacity for such reactions.<sup>9</sup>

## 3.3 Soluble protein content

*S. nepalense* contains 54.1±3.4 µg/gDW, *M. Maxalis rheedii* has 52.8 µg/gDW, and *C. sylvatica* has 48.9 µg/gDW. Proteins are essential for numerous biological functions, including enzyme activity, cellular repair, and growth. The higher protein content in *S. nepalense* indicates it could be a more valuable source of dietary protein or for use in protein extraction processes.<sup>10</sup>

## 3.4 Amino acids

*S. nepalense* registers the highest concentration at 98.6±3.5 µg/gDW, followed by *C. sylvatica* at 94.9 µg/gDW, and *M. Maxalis rheedii* at 91.3 µg/gDW. Amino acids are the building blocks of proteins and are critical for various metabolic pathways. The higher amino acid content in *S. nepalense* suggests it could be more beneficial in dietary supplements or therapeutic applications where amino acid enrichment is desired. Overall, *S. nepalense* consistently shows higher values across all measured phytochemicals, indicating its superior biochemical profile compared to the other two orchid species.<sup>11</sup>

### 3.5 Total Phenol

*Satyrium nepalense* exhibits the highest concentration of total phenolic compounds (29.6 mgGAE/gDW), followed by *M. rheedii* (24.7 mgGAE/gDW) and *C. sylvatica* (20.4 mgGAE/gDW). Total phenol content is responsible for antioxidant properties, and contribute significantly to the prevention of oxidative stress-related diseases by neutralizing free radicals.<sup>12</sup> They also exhibit anti-inflammatory, antimicrobial, and anticancer properties.<sup>13</sup> our study suggests that *Satyrium nepalense* may possess stronger antioxidant properties compared to the other two species.

### 3.6 Total Flavonoid

*C. sylvatica* has the highest concentration of total flavonoids (2.43 mg QE/g dry weight), followed by *M. rheedii* (2.49 mg QE/g dry weight) and *Satyrium nepalense* (1.89 mg QE/g dry weight). Flavonoids, a specific group of phenolic compounds, further enhance these benefits through their ability to modulate cell signaling pathways, inhibit enzymes involved in free radical production, and chelate metal ions that catalyze oxidative reactions.<sup>14,15</sup> Comparatively higher levels of *C. sylvatica* and *M. rheedii* might be responsible for their medicinal values.

### 3.7 Total Alkaloids

*Satyrium nepalense* has the highest concentration of total alkaloids (37.1 mg AE/g dry weight), followed by *C. sylvatica* (35.73 mg AE/g dry weight) and *Malaxis rheedii* (31.8 mg AE/g dry weight). Alkaloids often have diverse pharmacological activities, and their presence in these orchid species may indicate potential medicinal properties.<sup>16,17</sup>

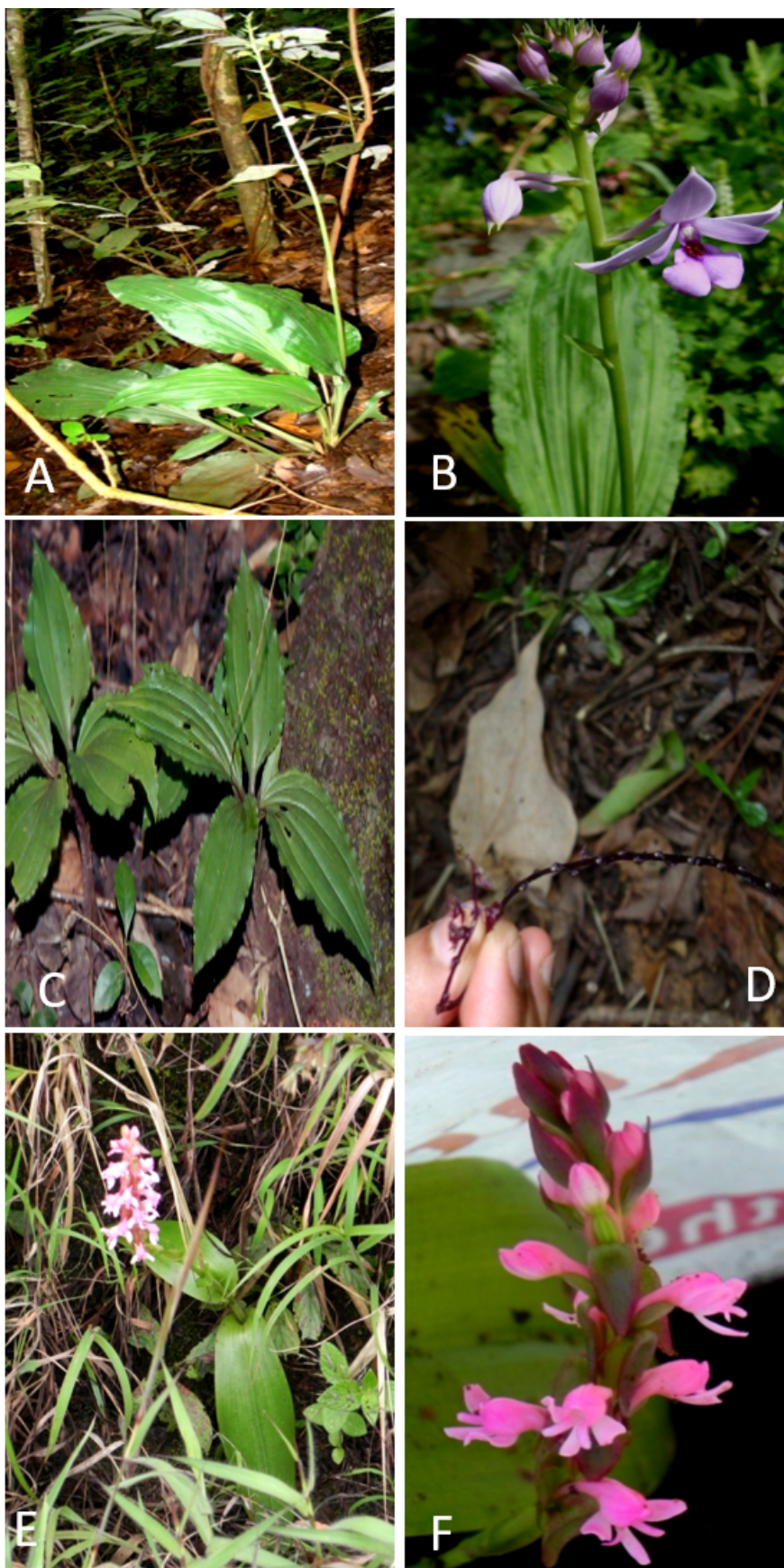
**Table 1:** Biochemical composition of *C. sylvatica*, *M. rheedii*, and *S. nepalense*.

Species	Total soluble sugar (µg/gDW)	Reducing sugar (µg/gDW)	Soluble protein (µg/gDW)	Amino acids (µg/gDW)
<i>C. sylvatica</i>	136.7±4.1	182.8±7.1	48.9±5.0	94.9±4.0
<i>M. rheedie</i>	140.8±5.0	184.6±5.6	52.8±4.2	91.3±4.7
<i>S. nepalense</i>	144.2±3.8	186.5±6.2	54.1±3.4	98.6±3.5

**Table 2:** Phytochemical composition of *C. sylvatica*, *M. rheedii*, and *S. nepalense*.

Species	Total phenol (mg GAE/gDW)	Total Flavonoid (mg QE/g dry weight)	Total Alkaloids (mg AE/g dry weight)
<i>C. sylvatica</i>	20.4±3.6	2.43±0.03	35.73±2.4
<i>M. rheedie</i>	24.7±2.0	2.49±0.15	31.8±3.2
<i>S. nepalense</i>	29.6±3.3	1.89±0.02	37.1±2.4

**Figure 1:** *C. sylvatica* (A, B), *M. rheedii*, (C, D) and *S. nepalense* (E, F) in their natural habitat



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