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Phytochemical Screening and Bioactive Compound Estimation of *Colebrookea oppositifolia*

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Abstract: ***Colebrookea oppositifolia*, a traditionally valued medicinal plant, was investigated for its phytochemical constituents using different solvent extracts. The aerial parts of the plant were shade-dried, powdered, and subjected to sequential extraction using n-hexane, chloroform, ethyl acetate, and methanol. Preliminary phytochemical screening revealed a wide distribution of secondary metabolites, with methanol extract showing the highest diversity, including alkaloids, flavonoids, tannins, saponins, phenolics, and glycosides. Chloroform and n-hexane fractions were particularly rich in terpenoids and steroids, while ethyl acetate extract demonstrated moderate amounts of flavonoids and phenolic compounds. These findings confirm that solvent polarity plays a critical role in the extraction of specific phytochemicals. The results provide a scientific basis for the plant's traditional therapeutic applications and point to its potential as a source of bioactive compounds for future pharmacological research. Further studies, including compound isolation and bioactivity assays, are recommended to explore its full medicinal potential.**

Keywords: *Colebrookea oppositifolia, Phytochemical Screening, Solvent Extraction, Secondary Metabolites, Alkaloids, Terpenoids.*

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I. INTRODUCTION

Medicinal plants continue to serve as an indispensable source of therapeutic compounds, particularly in developing countries where traditional knowledge still guides a large proportion of primary healthcare. According to the World Health Organization (WHO), approximately 80% of the world's population relies on plant-based remedies for their basic health needs (1, 2). In this context, *Colebrookea oppositifolia* Sm., a perennial shrub belonging to the family Lamiaceae, has emerged as a plant of considerable interest due to its diverse ethnomedicinal uses and wide geographical distribution across the Indian subcontinent, especially in hilly and forested regions of India, Nepal, and Bhutan (3).

C. oppositifolia has long held a place in traditional healing systems. In Ayurveda and folk medicine, the leaves and roots are used in the treatment of fever, epilepsy, cough, wounds, and various inflammatory conditions (4). Tribal communities often apply fresh leaf paste to cuts and wounds, or use decoctions for respiratory and gastrointestinal issues. Despite its widespread use, the plant has received limited attention in mainstream phytochemical research. A few studies have suggested the presence of secondary metabolites such as flavonoids, terpenoids, alkaloids, and saponins, but comprehensive profiling—particularly with solvent-based comparisons and quantitative estimations—remains largely unreported.

Phytochemicals such as phenolics and flavonoids are secondary plant metabolites known for their potent antioxidant, anti-inflammatory, antimicrobial, and anticancer activities (5). These compounds scavenge reactive oxygen species (ROS), reduce oxidative stress, and modulate cellular signalling pathways involved in inflammation and carcinogenesis (6). In plants like *C. oppositifolia*, which are used traditionally for treating inflammatory and infectious conditions, it is likely that these metabolites are key contributors to bioactivity. However, such assumptions require validation through systematic phytochemical analysis and quantification.

Recent studies have started to highlight the pharmacological potential of *C. oppositifolia*. A 2023 study by Peron et al. reported that methanolic leaf extracts exhibited significant antioxidant activity in DPPH and FRAP assays, correlating with a high concentration of phenolic compounds (3). Another investigation demonstrated antimicrobial synergy between essential oils of *C. oppositifolia* and conventional antibiotics against *Staphylococcus aureus*, indicating potential applications in combating drug-resistant bacteria (7). Furthermore, a 2021 LC-MS/MS-based study identified bioactive constituents such as acteoside, apigenin,

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and chrysin derivatives—flavonoids and phenylethanoid glycosides that are known for their anti-inflammatory and neuroprotective roles (8).

While these advanced analytical studies offer valuable insights, there is still a pressing need to conduct more accessible, standardized methods of phytochemical screening—such as solvent-specific extraction followed by classical tests and colorimetric quantification. Such approaches are especially valuable in early-stage research where access to advanced instrumentation may be limited but basic screening can help prioritize extracts for bioactivity testing. Quantifying total phenolic content (TPC) and total flavonoid content (TFC) using methods like Folin–Ciocalteu and aluminum chloride assays respectively is well-established and yields reproducible data (9) (Singleton et al., 1999; Kumari & Jain, 2020).

Solvent selection plays a crucial role in phytochemical extraction. Polar solvents like methanol and ethanol are known to extract more phenolic and flavonoid compounds, whereas aqueous and chloroform-based solvents may yield more glycosides, tannins, or non-polar compounds. A comparative analysis using multiple solvents can thus provide a clearer picture of phytochemical diversity and potential biological activity. This approach is particularly relevant to *C. oppositifolia*, where traditional preparations often rely on water-based decoctions, but modern pharmacology may benefit more from alcohol-based extractions.

Moreover, environmental factors such as altitude, climate, and season can influence the concentration of phytochemicals in plants. For a widely distributed species like *C. oppositifolia*, documenting the chemical profile from specific geographic sources contributes not only to scientific understanding but also to the development of region-specific formulations in traditional and modern medicine.

The present study was designed to address existing gaps in the phytochemical evaluation of *Colebrookea oppositifolia*. The main objectives are to: (1) qualitatively identify the major phytochemical classes present in different solvent extracts of the plant; (2) quantitatively determine the total phenolic and flavonoid content in those extracts; and (3) compare the phytochemical richness across solvents to suggest the most suitable extract for further biological investigation.

By using standard extraction procedures and validated analytical protocols, this research aims to lay the groundwork for more detailed pharmacological assessments in future. The findings are expected to not only validate the traditional use of *C. oppositifolia* but also enhance its prospects as a source of natural therapeutic agents, particularly in antioxidant and anti-inflammatory drug development.

II. MATERIALS AND METHODS

➢ *Plant Collection and Authentication*

The aerial parts (flowers, leaves and stems) of C. oppositifolia were sourced from foothills of the Siswan Reserve Area located near Baddi, H.P., at coordinates 30°52′10.28 N latitude, 76°44′45.59 E longitude in February. Dr. Kailash C. Bhatt, Principal Scientist at ICAR-NBPGR, New Delhi, India, authenticated the plant material. The collected plant specimen was archived at Department of Herbarium in Botanical Survey of India (BSI) with accession number AC-242/2024. Authentication certificate was shown in **Error! Reference source not found.**

➢ *Preparation of Plant Material*

The freshly collected aerial parts of the plant were first air-dried in a shaded, well-ventilated area at room temperature (approximately 25°C). This drying process was continued for about two weeks until the plant material reached a consistent, stable weight. Once fully dried, the material was finely ground into powder using a mechanical grinder to ensure uniform particle size for extraction. A total of 3.2 kilograms of this powdered plant matter was then subjected to maceration in 10 liters of dichloromethane (DCM), allowing the solvent to thoroughly interact with the plant matrix and extract its bioactive constituents (10).

The maceration was carried out in a sealed container at ambient room temperature over a period of 72 hours. During this time, the mixture was gently shaken at regular intervals to enhance solvent penetration and improve the extraction efficiency of phytochemical constituents from the plant matrix. After maceration, the entire content was passed through Whatman No. 1 filter paper to separate the plant residue from the solvent extract. The resulting filtrate was then concentrated under reduced pressure using a rotary evaporator, operated at 40°C, to gently remove the solvent without degrading heat-sensitive compounds. This process yielded the semi-solid crude extract, which was then stored for further phytochemical analysis (10).

The resulting crude plant extract (COCP) was weighed to determine the amount obtained and stored in an airtight amber glass container at 4°C in the dark to prevent degradation of light-sensitive compounds until further analysis.

➢ *Fractional Extracts Preparation*

Four fractionated extracts were obtained from the crude plant extract with increasing polarity solvents: n-hexane, chloroform, ethyl acetate, and methanol. First, 750 g of crude extract was dissolved in 700 mL of distilled water and transferred to a separatory funnel. This aqueous fraction was successively partitioned by extraction with 300 mL of n-hexane (three times), then chloroform (three times), and ethyl acetate (three times). The remaining aqueous layer was then concentrated to obtain the methanol fraction **(11)** The obtained fractions such as n-hexane fraction (COHE), chloroform fraction (COCH), Ethyl acetate fraction (COEA), and methanol fraction (COMT) were dried over anhydrous sodium sulfate, and concentrated at reduced pressure at 40°C using a rotary evaporator. Crude and all fractional extracts were kept in amber glass vials at -20°C until later use **(12).**

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➢ *Preliminary Phytochemical Screening*

The preliminary phytochemical analysis of various solvent fractions of *Colebrookea oppositifolia* was conducted to identify the major classes of secondary metabolites present in the plant. Four different solvent extracts—methanol, n-hexane, chloroform, and ethyl acetate—were screened using established qualitative tests. Each solvent system was selected based on its polarity, ranging from non-polar (n-hexane) to highly polar (methanol), to ensure the broadest possible range of phytochemical extraction. The tests were carried out using standard procedures as described by Harborne (1998) with slight modifications to adapt to the laboratory conditions (10, 13).

➢ *Alkaloids*

To test for alkaloids, small portions of each extract were treated with Dragendorff's reagent and Mayer's reagent separately. The appearance of an orange-brown precipitate with Dragendorff's reagent or a cream-colored precipitate with Mayer's reagent was considered indicative of alkaloid presence. Alkaloids were prominently detected in the methanol extract, while being absent in n-hexane and only weakly present in the ethyl acetate and chloroform fractions.

➢ *Flavonoids*

The presence of flavonoids was determined by the alkaline reagent test. A yellow coloration that turned colorless upon the addition of dil. acid confirmed flavonoid presence. Flavonoids were strongly present in the methanol and ethyl acetate fractions. In contrast, the n-hexane and chloroform extracts exhibited only trace or no flavonoid content.

➢ *Tannins*

For tannins, extracts were treated with ferric chloride solution. A blue-black or greenish-black coloration indicated the presence of hydrolyzable or condensed tannins, respectively. Tannins were clearly observed in the methanol extract, with moderate reaction in the ethyl acetate fraction. Chloroform and n-hexane extracts did not show noticeable tannin presence.

➢ *Saponins*

The froth test was employed to assess saponin content. Each extract was diluted with distilled water and vigorously shaken. Persistent frothing lasting more than 15 minutes suggested saponins. Among the tested solvents, only the methanol extract showed a significant froth layer, indicating the presence of saponins, while the other fractions were negative.

➢ *Terpenoids*

To detect terpenoids, the Salkowski test was performed. The addition of chloroform followed by concentrated sulfuric acid resulted in a reddish-brown interface in positive samples. Chloroform and n-hexane fractions showed a positive reaction, suggesting that these non-polar and semi-polar solvents were effective in extracting terpenoid compounds. Methanol and ethyl acetate extracts showed minimal or no terpenoid presence.

➢ *Steroids*

Steroids were screened using the Liebermann–Burchard reaction. A greenish-blue color change following the addition of acetic anhydride and concentrated sulfuric acid was considered positive. Steroids were notably present in the chloroform and n-hexane extracts, with weak or absent signals in the polar solvents.

➢ *Glycosides*

To test for glycosides, the Keller–Killiani test was used. A reddish-brown ring at the junction of two layers after reaction indicated a positive result. Methanol extract showed strong positivity for glycosides, while ethyl acetate extract revealed a moderate reaction. The n-hexane and chloroform fractions showed no significant color change.

➢ *Phenolic Compounds*

Phenolic compounds were tested using ferric chloride and lead acetate tests. A deep blue or green coloration with ferric chloride and white precipitate with lead acetate indicated the presence of phenolics. Phenolic compounds were markedly present in the methanol extract and moderately present in ethyl acetate. No reaction was observed in n-hexane and chloroform extracts.

III. RESULTS AND DISCUSSION

➢ *Collection and Authentication*

The aerial parts of the plant was used for crude plant preparation, which was depicted in Fig 1. The plant was collected in February. The herbarium submitted for authentication was illustrated in Fig 2.

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Fig 1 Aerial Parts of Colebrookea Oppositifolia

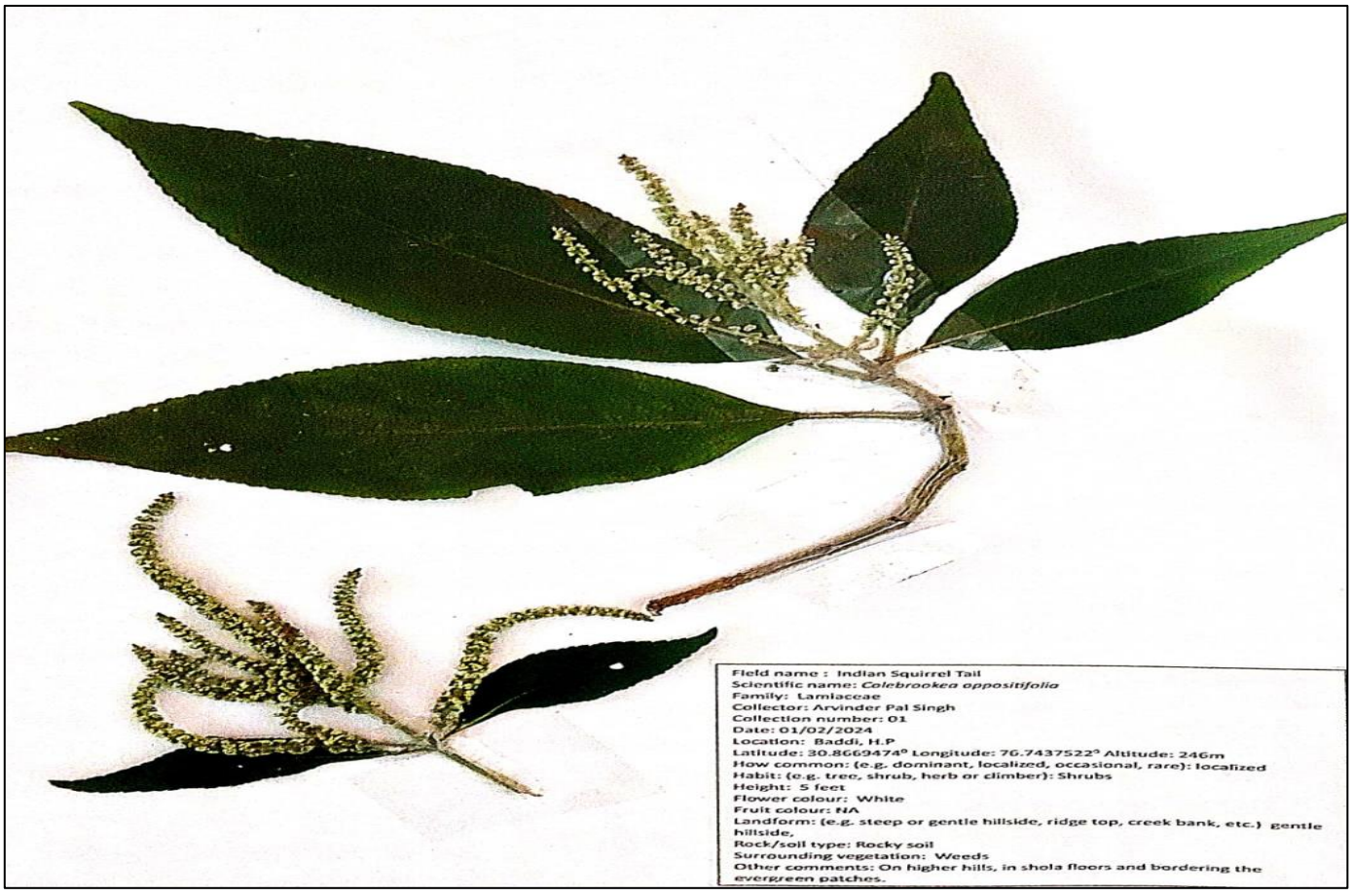

Fig 2 Herbarium of Colebrookea Oppositifolia

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➢ *Phytochemical Screening:*
Various phytochemicals detected in the fractions of *Colebrookea oppositifolia* were shown in

Table *1*.

Table 1 Phytochemicals detected

Phytochemical Group	Methanol	Ethyl acetate	Chloroform	n-hexane
Alkaloids	+++	+	+	–
Flavanoids	+++	++	–	–
Tannins	++	+	–	–
Saponins	++	–	–	–
Terpenoids	–	+	++	++
Steroids	–	–	++	++
Glycosides	++	+	–	–
Phenolics	+++	++	–	–

(+++) = Strong presence; (++) = Moderate; (+) = Trace; (–) = Absent

The preliminary phytochemical screening of Colebrookea oppositifolia revealed a distinct distribution of secondary metabolites across different solvent fractions. Methanol extract showed the broadest phytochemical profile, with strong presence of alkaloids, flavonoids, phenolics, tannins, saponins, and glycosides. This aligns with the high polarity of methanol, which effectively solubilizes a wide range of polar phytochemicals. Ethyl acetate, being moderately polar, extracted compounds like flavonoids, phenolics, and glycosides, though in lesser intensity. In contrast, non-polar solvents like n-hexane and chloroform were more effective in extracting terpenoids and steroids, which are typically lipophilic in nature. The absence of certain phytochemicals in non-polar extracts emphasizes the importance of solvent selection in phytochemical studies. These findings support the traditional use of this plant in folk medicine and provide a basis for further phytopharmacological investigations. The solvent-specific variation in phytoconstituents suggests that targeted extraction can enhance the yield of specific bioactive groups for therapeutic exploration.

IV. CONCLUSION

The current study provides a foundational understanding of the phytochemical composition of *Colebrookea oppositifolia*, with a focus on solvent-based extraction and screening. The results clearly demonstrate that the choice of solvent significantly influences the range and type of phytochemicals extracted from the plant. Among the tested solvents, methanol proved to be the most effective in extracting a broad spectrum of bioactive compounds, including alkaloids, flavonoids, tannins, saponins, phenolics, and glycosides. This suggests that methanolic extracts of *C. oppositifolia* may hold considerable pharmacological potential, particularly for antioxidant, antimicrobial, and anti-inflammatory activities, which are commonly associated with these phytochemical groups. Chloroform and n-hexane extracts, while less diverse, were rich in terpenoids and steroids—compounds often linked with antimicrobial and anticancer properties. The moderate presence of flavonoids and phenolics in the ethyl acetate extract further highlights its value for antioxidant studies.

These findings underscore the therapeutic promise of *C. oppositifolia*, validating its traditional uses and supporting further research into its pharmacological activities and compound isolation for drug development.

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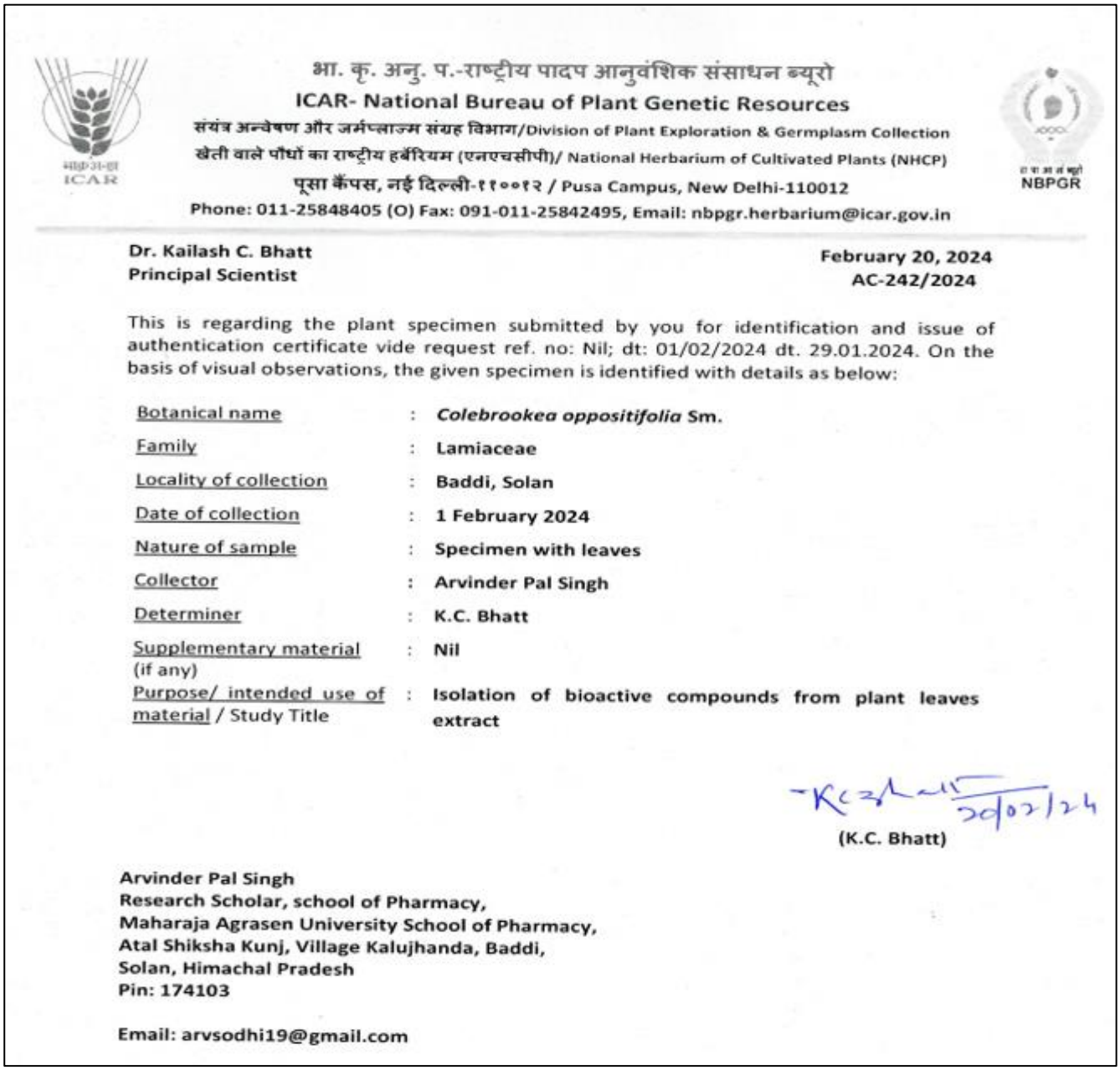

भा. कृ. अनु. प.-राष्ट्रीय पादप आनुवंशिक संसाधन ब्यूरो
ICAR- National Bureau of Plant Genetic Resources
संयंत्र अन्वेषण और जर्मप्लाज्म संग्रह विभाग/Division of Plant Exploration & Germplasm Collection
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NBPGR

Dr. Kailash C. Bhatt
Principal Scientist

February 20, 2024
AC-242/2024

This is regarding the plant specimen submitted by you for identification and issue of authentication certificate vide request ref. no: Nil; dt: 01/02/2024 dt. 29.01.2024. On the basis of visual observations, the given specimen is identified with details as below:

Botanical name	:	*Colebrookea oppositifolia* Sm.
Family	:	Lamiaceae
Locality of collection	:	Baddi, Solan
Date of collection	:	1 February 2024
Nature of sample	:	Specimen with leaves
Collector	:	Arvinder Pal Singh
Determiner	:	K.C. Bhatt
Supplementary material (if any)	:	Nil
Purpose/ intended use of material / Study Title	:	Isolation of bioactive compounds from plant leaves extract

20/02/24
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Fig 3 Autherntication Certificate