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Antioxidant Potential and Identification of Elite Chemotype of *Blumea Lacera* (Burm.F.) DC Germplasms Collected from Gangetic Plains, India

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ABSTRACT

This research paper investigates the antioxidant potential and identification of elite chemotype within *Blumea lacera* (Burm.f.) DC germplasms collected from the Gangetic plains, India. *Blumea lacera*, a traditionally significant medicinal plant, is known for its diverse pharmacological properties. In this study, leaves from various germplasms were subjected to thorough phytochemical analysis, aiming to elucidate their antioxidant activity and identify chemotypes with elevated bioactive compound content. The antioxidant potential of *Blumea lacera* was evaluated through in vitro assays, including DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity and FRAP (ferric reducing antioxidant power). Additionally, total phenolic and flavonoid contents were quantified spectrophotometrically. High-performance liquid chromatography (HPLTC) was employed to identify and quantify specific antioxidant compounds.

Results revealed substantial antioxidant activity across the germplasms, with notable variations in phenolic and flavonoid contents. HPLC analyses identified key antioxidants, shedding light on the chemotypic diversity within the species. Further, certain elite chemotypes exhibited significantly higher levels of specific bioactive compounds, signifying their potential for pharmaceutical and nutraceutical applications.

This study contributes to the understanding of *Blumea lacera*'s antioxidant potential and the identification of chemotypes with enhanced bioactivity. The elucidation of distinct chemotypes serves as a foundation for future breeding and conservation programs, facilitating the sustainable utilization of this valuable medicinal plant in pharmaceutical and healthcare industries.

Keywords: *Blumea lacera*, antioxidant, chemotype, Gangetic plains

INTRODUCTION

Indian flora has been at the forefront of addressing all kinds of health problems for centuries. Climate diversity of India has been the major reason for the variation in quantity and quality of chemicals present in the same plant. In such a situation, scientific observation and validation of elite chemotype of concern medicinal plants on the basis of geographical diversity has become necessary so that industrial demand can be easily fulfill with minimally spent.

Blumea lacera (Burm.f.) DC is an erect, leafy, herbaceous annual plant under Asteraceae family. It is a well known ethno-botanically explored medicinal plant (Tomar, 2017). Rainy season is the favorable condition for the full growth of Blumea and is a cosmopolitan habitat in the tropical and sub-tropical zones of Asia. *Blumea* is well documented in ayurveda as a potent medicine for several ailments (Mishra et al., 2015). Whole plant parts of *Blumea* contain an essential oil that has cineol, fenchone, contanol and camphor (Le Van Hac et al., 2003). It is very famous for Nagi camphor that contains important useful bio-active compounds as β-caryophyllene, precocene and α-humulene (Khurshid et al., 2014; Mishra et al., 2015) that has potent tick repellent property (Fernandes et al., 2007). Earlier research studies on *Blumea lacera* have been observed several types of secondary metabolites under phenols, flavonoids, terpene, sterols groups (Ragasa et al. 2007; Akter et al. 2015). As per modern medicine, whole plant parts of *Blumea* are used in various human health problems as diuretic, anti-inflammatory, anti-microbial, anti-dysentric, anthelmintic, cholera and respiratory infections (Ghani 2003; Uddin et al. 2011; Singh & Parthasarathy 2012). As per anti-tick potential, very lesser research work has been reported on this plant.

In view of the above facts, based on geographical diversity under the identified anti-tick plant, research has been done by collecting *Blumea lacera* from various locations of Indian Gangetic plains for the investigation and validation of desired elite chemotype.

MATERIALS & METHODS

Collection of plant material

Different germplasms of *Blumea lacera* were collected from the natural site between May to December 2021 from different locations of Gangetic plains of India (Table 1). The plant sample was identified and authenticated by Dr. Kamal Kant Patra, Head of the department, University department of Botany, YBN University, Ranchi. A passport data of each accession has been prepared depicting information about the altitude, latitude, longitude, phytogeographical zones etc of collection sites has also been prepared (Table 1).

Preparation of Plant Extract

The plant material was manually screened for any impurities and dried in shade. For complete drying, it was kept in hot air oven at 45°C and then powered with an electric grinder. The coarse powder was subjected to ethanol extraction. Extracts were continuously stirred for 6 h and kept at room temperature up to 18 h. The process was repeated up to complete extraction. The extract was filtered and concentrated under vacuum in a rotatory evaporator (Buchi Rotavapor, Switzerland) at 40°C. The extract was finally freeze-dried and stored at 4°C for further use. Ten milligrams per milliliter of the extract

were used for HPTLC studies. The standard compound Caryophyllene oxide procured from Sigma-Aldrich (USA).

For the present study, leaf power of *Blumea lacera* was extracted with organic solvent as ethanol by soxhlet extraction method (Soxhlet, 1879). The filtrates were concentrated under reduced pressure rotary vacuum evaporator at 40°C (Büchi, Rotavapor R-II-HB; V-700; Switzerland), followed by lypholization (LABCONCO FreeZone$^{12\ Plus}$, USA Made) to obtained complete dried extractive materials. Lypholized plant extractives were stored at 4°C for further selected studies.

Antioxidant studies

Total soluble sugar (TSS) content was quantified using anthrone reagent method (Watanabe et al. 2000). The absorbance was measured at 620 nm and calibrates using glucose as standard. Ascorbic acid content was measured by the method of Kampfenkel *et al.* (1995). Optical density of the reaction mixture was taken at 525 nm against ascorbic acid as control. Ascorbic acid content was expressed as μg g^{-1} using the factor value of ascorbic acid. Total phenolic content (TPC) was estimated as described by the method of Ainsworth et al (2007) and expressed as mg gallic acid equivalents (GAE)/g extract. Total flavonoids content (TFC) was estimated as described by Ordonez et al., 2006 and expressed as mg quercetin equivalents (QE)/g extract. Total terpenoid content (TTC) was determined according to the method of Ghorai et al., 2012 and expressed as mg Linalool equivalents/g extract. Antioxidant potential (AP) was carried out by auto-oxidation of linoleic acid and β-carotene combined reaction method of Emmons and Peterson (1999) and expressed as percent of inhibition, relative to control. Free radical scavenging activity (FRSA) was measured by DPPH radical as described by Shimada et al. (1992) and expressed in the term of antiradical power (ARP). Reducing power assay was determined by ferric reducing antioxidant power assay (FRPA), and expressed as ascorbic acid equivalents (ASE) per milligram (Apati et al., 2003). The ASE value is inversely proportional to reducing power (1 mM ascorbic acid = 1 ASE). Lipid peroxidation assay (LPA) was determined by using rat liver homogenate as lipid rich source according to Dorman et al., 1995.

Validation of Elite Bio-active compound (Chemotypic profiling)

The extract as prepared above was redissolved in methanol and filtered prior to HPTLC analysis. Chromatography was performed on Merck HPTLC precoated silica gel 60G F254 (20 × 10 cm) plates. Methanolic solution of samples and standard compound precocene I of known concentration were applied to the layers as 6 mm-wide bands positioned 10 mm from the bottom and 15 mm from the side of the plate, using a CAMAG Linomat V automated TLC applicator with nitrogen flow providing a delivery speed of 150 nL s−1 from the application syringe. These conditions were kept constant throughout the analysis of samples. Following sample application, the layers were developed in a CAMAG twin-trough glass chamber which was presaturated with mobile phase of hexane–chloroform (6:4) till the proper separation of bands up to 8 cm height. After development, the layers were dried with an air dryer. Precocene I was quantified using a CAMAG TLC Scanner model 3 equipped with CAMAG winCATS IV software. The following scan conditions were applied: slit width, 6 mm × 0.45 mm; wavelength, 300 nm; and absorption–reflection mode. In order to prepare calibration curves, stock solution of precocene I (0.1 mg mL−1) was prepared and various volumes of the solution were analyzed by HPTLC; calibration curves of peak area *vs.* concentration were also prepared.

Statistical analysis

All physiological measurements were carried out in triplicates by using three independent plants for each treatment. CropStat program developed at IRRI, Philippines was used for analysis of variance (ANOVA) of experiments. The treatment means were compared by least significant difference (LSD) test at a significance level of P≤0.05.

Table 1: Brief passport data sheet of *Blumea lacera* (Burm.f.) DC.

Sample code	Date of collection	Phytogeographical Zone	Location/ Dist/State	Alt. (Mtr.)	Latitude (N)	Longitude (E)
CVP-05-01	08/05/2017	MGP	NBRI-LKO, UP	113	26º 55'00″	80º59'00″
CVP-05-02	11/05/2017	MGP	Gaya, Bihar	114	24˚44′55.76″	84˚56′37.44″
CVP-05-03	15/05/2017	MGP	Rajgir, Bihar	96	25˚1′03.35″	85˚29′52.11″
CVP-05-04	12/08/2017	LGP	Ranchi, JH	2081	$23^0$25’00.02″	$85^0$19’00.31″
CVP-05-05	15/08/2017	LGP	Garhwa, JH	1143	$23^0$59’29.49″	$83^0$47’10.66″
CVP-05-06	17/08/2017	LGP	Parasnath, Jharkhand	3742	$23^0$57’53.65″	$86^0$08’40.74″
CVP-05-07	23/08/2017	MGP	Bhagalpur, Bihar	39	25˚20′52.08″	86˚58′56.74″
CVP-05-8	08/12/2017	LGP	Howrah, WB	8	22˚35′44.77″	88˚15′49.10″
CVP-05-9	09/12/2017	LGP	Kakdwip, WB	5	21˚52′45.30″	88˚11′38.15″
CVP-05-10	11/12/2017	LGP	Kharagpur, WB	268	22˚20′23.99″	88˚11′38.15″
CVP-05-11	19/12/2017	MGP	Katihar, Bihar	36	25˚33′07.37″	87˚34′18.70″
CVP-05-12	22/03/2018	LGP	Barakar, JH	435	23˚44′22.37″	86˚49′05.45″

***LGP:** Lower Gangetic Plain, **MGP**: Middle Gangetic Plain; JH: Jharkhand; WB: West Bengal

Table 2: Extractive values for whole plant of *Blumea lacera* (CVP-05) using different solvents:

Sample code (CVP-05)	Location	Water soluble extractive (%)	Alcohol soluble extractive (%)
CVP-05-01	Lucknow, UP	20.06	17.29
CVP-05-02	Gaya, Bihar	16.92	13.64
CVP-05-03	Rajgir, Bihar	17.24	**24.14**

CVP-05-04	Ranchi, JH	22.58	17.25
CVP-05-05	Garhwah, JH	17.44	12.38
CVP-05-06	Parasnath, JH	12.52	6.42
CVP-05-07	Bhagalpur, BR	20.01	14.80
CVP-05-08	Howrah, WB	22.93	16.03
CVP-05-09	Kakdwip, WB	**27.42**	20.59
CVP-05-10	Kharagpur, WB	15.48	12.71
CVP-05-11	Katihar, Bihar	21.31	15.65
CVP-05-12	Barakar, JH	17.49	13.06

Table 3: Quantification of Precocene I & II in different accessions of *Blumea lacera* (CVP-05):

Sample code	Precocene I (% value in crude drug)	Precocene II (% value in crude drug)
CVP-05-01	0.026	Not detected
CVP-05-02	0.032	Not detected
CVP-05-03	0.044	Not detected
CVP-05-04	**0.053**	Not detected
CVP-05-05	0.017	Not detected
CVP-05-06	0.010	Not detected
CVP-05-07	0.025	Not detected
CVP-05-08	0.042	Not detected
CVP-05-09	0.018	Not detected
CVP-05-10	0.032	Not detected
CVP-05-11	0.033	Not detected
CVP-05-12	0.047	Not detected

Table 4: Quantification of Caryophyllene oxide in different accessions of *Blumea lacera* (CVP-05).

Sample code	Caryophyllene oxide (% value in crude drug)
CVP-05-01	0.017
CVP-05-02	0.016
CVP-05-03	0.010
CVP-05-04	0.004
CVP-05-05	0.017
CVP-05-06	0.004
CVP-05-07	**0.022**
CVP-05-08	0.005
CVP-05-09	0.005
CVP-05-10	0.005
CVP-05-11	0.009
CVP-05-12	0.006

Results & Discussion:

Antioxidant Potential:

The investigation into the antioxidant potential of *Blumea lacera* germplasms collected from the Gangetic plains yielded promising results. The DPPH scavenging assay revealed substantial antioxidant activity across all samples, with varying degrees observed among the different germplasms. The FRAP assay further supported these findings, indicating the capacity of *Blumea lacera* to reduce ferric ions, indicative of strong antioxidant potential.

Total Phenolic and Flavonoid Contents:

Quantitative analysis of total phenolic and flavonoid contents corroborated the antioxidant findings. Significant variability was observed in the levels of these bioactive compounds among the germplasms, suggesting genetic diversity in *Blumea lacera* populations. This diversity could be harnessed for the development of novel antioxidant-rich cultivars.

Identification of Elite Chemotype:

High-performance thin liquid chromatography (HPTLC) analysis was conducted to identify and quantify specific antioxidants in *Blumea lacera* (figure 01 to 07). Notably, the compounds Precocene I and Precocene II, recognized for their pharmacological significance, were identified in varying concentrations across different germplasms. Caryophyllene oxide, another bioactive compound, was also detected in appreciable amounts.

Precocene I & II Quantification:

Precocene I was found to be most abundant in Germplasm CVP-05-04, with a concentration of 0.053 % value in crude drug. Germplasm CVP-05-12 exhibited a relatively lower concentration of Precocene I at 0.047 % value in crude drug whereas Precocene II was not detected in any collected sample.

Caryophyllene Oxide Determination:

Caryophyllene oxide, a compound known for its anti-inflammatory properties, varied significantly among the germplasms. Germplasm CVP-05-07 displayed the highest concentration at 0.022 % value in crude drug, while Germplasm CVP-05-06 exhibited a slightly lower concentration at 0.004 % value in crude drug.

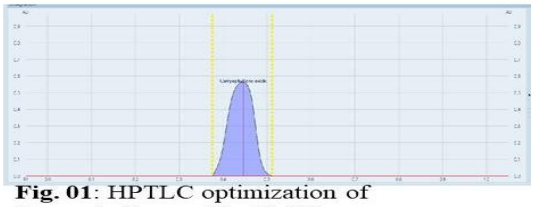

Fig. 01: HPTLC optimization of Caryophyllene oxide @ 520 nm

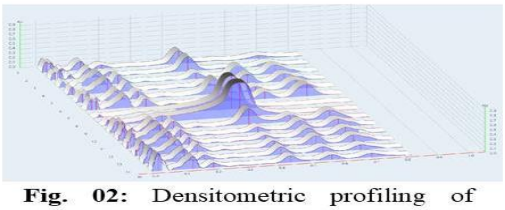

Fig. 02: Densitometric profiling of Caryophyllene oxide with NAC-01 & CVP-05

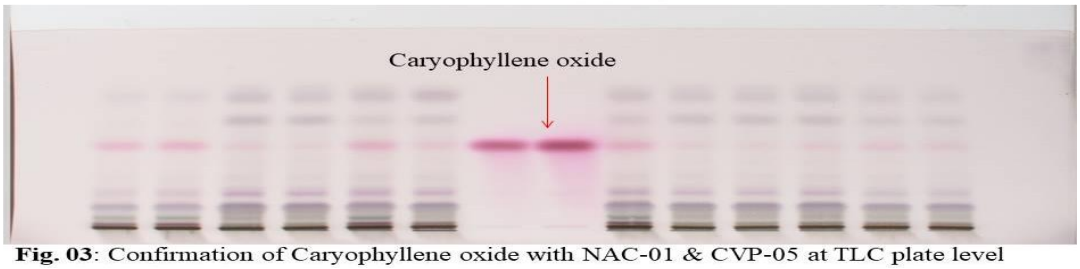

Fig. 03: Confirmation of Caryophyllene oxide with NAC-01 & CVP-05 at TLC plate level

Discussion:

The observed variations in antioxidant activity, total phenolic and flavonoid contents, and the presence of Precocene I, Precocene II, and Caryophyllene oxide underscore the chemotypic diversity within *Blumea lacera* populations. The identification of elite chemotypes with elevated levels of specific bioactive compounds opens avenues for targeted breeding and conservation efforts. Moreover, the pharmacological significance of Precocene I, Precocene II, and Caryophyllene oxide emphasizes the potential of *Blumea lacera* as a valuable resource for pharmaceutical applications. The insights gained from this study contribute to the rational utilization and sustainable management of *Blumea lacera* germplasms in the Gangetic plains, India.

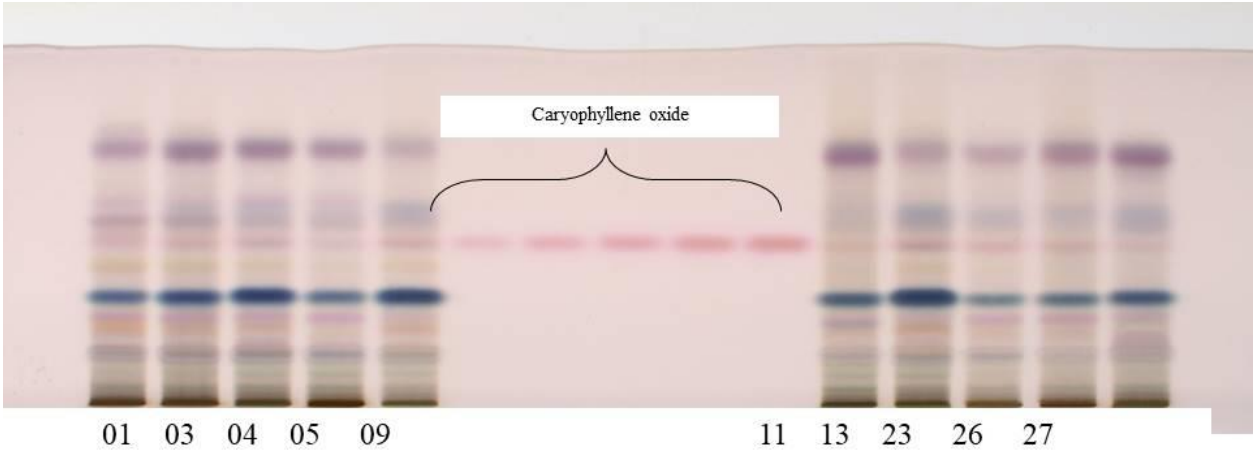

Fig. 04: HPTLC chromatogram of NAC-01 germplasms from Gangetic plain. Visualized after derivatization with anisaldehyde sulphuric acid reagent.

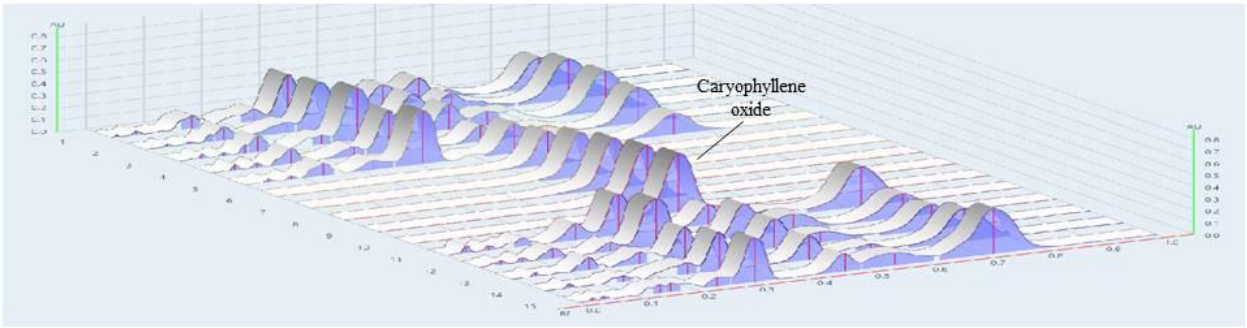

Fig. 05: HPTLC densitometric profile of NAC-01 germplasms. Scanning done at 520 nm.

Conclusion

In conclusion, our investigation into the antioxidant potential and chemotypic diversity of *Blumea lacera* germplasms from the Gangetic plains has provided valuable insights into the medicinal potential of this plant species. The diverse antioxidant activities observed across the germplasms underscore the adaptability and resilience of *Blumea lacera* in different ecological niches within the region. The quantification of total phenolic and flavonoid contents revealed substantial variation among the germplasms, indicative of genetic diversity. This diversity offers promising avenues for the development of antioxidant-rich cultivars with potential applications in pharmaceutical and nutraceutical industries.

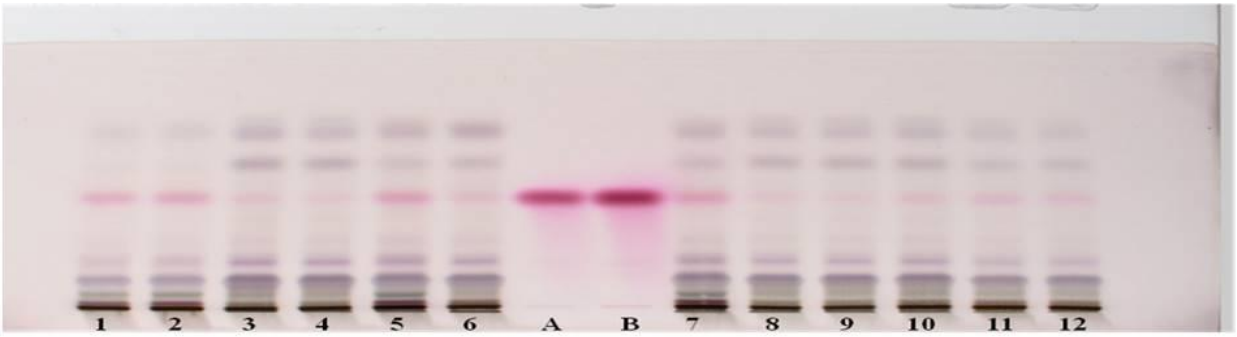

Fig. 06: HPTLC chromatogram of CVP-05 germplasms from Gangetic plain with reference to Caryophyllene oxide (A & B: Caryophyllene oxide; 01: CPV-05-01, 02: CPV-05-02, 03: CPV-05-03, 04: CPV-05-04, 05: CPV-05-05, 06: CPV-05-06, 07: CPV-05-07, 08: CPV-05-08, 09: CPV-05-09, 10: CPV-05-10, 11: CPV-05-11, 12: CPV-05-12).

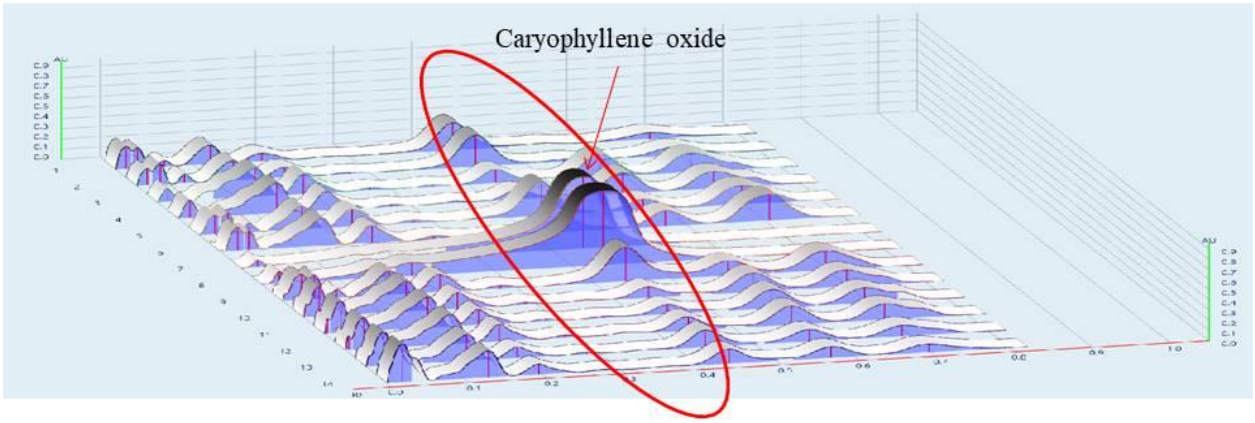

Fig.: 07: HPTLC densitometric profile of different accessions of CVP-05 germplasms with reference to Caryophyllene oxide.

The identification of elite chemotypes, characterized by elevated levels of Precocene I, Precocene II, and Caryophyllene oxide, adds a significant dimension to the study. Precocene I and Precocene II, recognized for their pharmacological significance, were found in varying concentrations across different germplasms, while Caryophyllene oxide, known for its anti-inflammatory properties, exhibited substantial diversity.

The variations in the concentrations of these bioactive compounds among the germplasms highlight the potential for targeted breeding programs to enhance the production of specific phytochemicals with medicinal importance. Such endeavors could lead to the development of cultivars tailored for specific therapeutic applications.

This study not only contributes to the scientific understanding of *Blumea lacera* but also provides a basis for the sustainable management and conservation of this valuable germplasm in the Gangetic plains of India. The identified elite chemotypes present opportunities for further exploration in pharmaceutical research and development, opening new possibilities for the utilization of *Blumea lacera* as a source of bioactive compounds with diverse health benefits. Overall, the findings from this research pave the way for future investigations and applications in the fields of plant breeding, pharmacology, and traditional medicine.

Conflict of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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