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GCMS-BASED COMPARATIVE ANALYSIS OF BIOACTIVE PHYTOCHEMICALS IN THE LEAVES OF SELECTED *Bougainvillea* SPECIES

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ABSTRACT
The current research work was done to compare and analyse the phytochemical constituents of leaf extracts of four varieties of *Bougainvillea* Torchglow, Chitra, Vishaka and Mahara, which had been grown in pots under semi-shaded conditions. A combination of sterile soil, coco peat, perlite, vermicompost and sand was used to grow plants in pots and maintained over three months. There was soil profiling to determine the state of the soil in terms of fertility. The phytochemical compounds of the leaf extracts were determined with the help of gas chromatography-mass spectrometry (GC-MS). Ethanol, methanol, and hexane were used to extract the metabolites to have a wide coverage. In total, 23 compounds were tentatively identified, and they belonged to various chemical classes such as phenolics, esters, fatty acids, terpenoids, hydrocarbons, amides, and long-chain alkanes. The phytochemical richness of methanolic extracts was the highest, with 9-12 compounds per variety, followed by hexane extracts with 4-7 compounds, whereas ethanolic extracts showed only one consistently detected compound, triethyl citrate (retention time 19.54-19.55 min), which is also a known plasticiser-related contaminant and is therefore interpreted with caution. Heat mapping-based comparative analysis showed that there were solvent- and variety-specific differences in the distribution of metabolites, with Mahara having the greatest total chemical diversity. The results demonstrate the powerful effect of the solvent polarity on the extraction efficiency and indicate the phytochemical richness of *Bougainvillea* leaves. The current study offers background information for the promising use of *Bougainvillea* species as the source of bioactive compounds that can find use in pharmaceuticals and industry. These findings also contribute to the development of plant-based bioactive resources, which supports the SDG 3, i.e., Good Health and Well-being.
Keywords: *Bougainvillea* Species, Bioactive compounds, GC-MS Analysis, Medicine, Phytochemicals, Soil Analysis, Fertility.
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INTRODUCTION

Plants have long served as a major source of bioactive compounds with significant pharmacological potential, largely due to their diverse secondary metabolites such as phenolics, flavonoids, terpenes, and fatty acids. These compounds are increasingly explored for their antioxidant, antimicrobial, anti-inflammatory, and therapeutic properties, making phytochemical profiling an essential step in identifying plant-based drug candidates.[1,2] The exploration of plant-derived bioactive compounds also aligns with Sustainable Development Goal (SDG) 3: Good Health and Well-being by supporting the discovery of natural therapeutic agents. Among ornamental plants, *Bougainvillea* species (family Nyctaginaceae) have gained attention not only for their aesthetic value but also for their traditional medicinal uses. *Bougainvillea* is widely distributed in tropical and subtropical regions and has been traditionally used to treat respiratory disorders, gastrointestinal ailments, inflammation, and microbial infections.[3] Recent

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studies have demonstrated that *Bougainvillea* species contain a rich array of phytochemicals, including alkanes, phenolics, terpenoids, fatty acids, and betalains, many of which exhibit antioxidant, antibacterial, anticancer, and anti-inflammatory activities.[4,5] Furthermore, GC–MS-based studies have identified key bioactive compounds such as phytol, hexadecanoic acid, and benzoic acid derivatives, which are associated with antimicrobial and antioxidant properties.[6] The efficiency of phytochemical extraction is strongly influenced by solvent polarity, which determines the type and quantity of compounds extracted. Polar solvents such as methanol and ethanol are generally more effective in extracting phenolics and flavonoids, whereas non-polar solvents like hexane preferentially isolate lipophilic compounds such as hydrocarbons and fatty acids.[1,7] Such solvent-dependent variability has been consistently reported across medicinal plants, where differences in extraction systems significantly alter both qualitative and quantitative phytochemical profiles and their associated bioactivities.[8] Gas chromatography–mass spectrometry (GC–MS) is a widely used analytical technique for the identification and characterisation of volatile and semi-volatile phytochemicals, enabling detailed profiling of complex plant extracts.[9] Its use in comparative phytochemical analysis offers invaluable information on compositional variations between plant species, varieties, and extraction techniques. Although there is increasing demand in *Bougainvillea*, there is less comparative research on assessing phytochemical diversity among various kinds of *Bougainvillea* using various solvents. Specifically, systematic GC-MS profiling of leaf extracts of individual cultivars can give more insight into the varietal differences and their possible biological relevance. These comparative studies are vital in the discovery of superior varieties of phytonutrients in a range of elites and their pharmaceutical applications. Thus, the current research paper will focus on researching and comparing phytochemical profiles of leaf extracts of four *Bougainvillea* cultivars using various solvents (methanol, ethanol, and hexane) as research tools by means of GC-MS. The aim of this work is to clarify the solvent-dependent differences in phytochemical profiles and characterise important bioactive compounds that could be the source of the pharmacological activity of these ornamental plants. The findings of this study provide a scientific foundation for selecting suitable extraction solvents and identifying phytochemically rich *Bougainvillea* cultivars for future pharmacological investigations.

EXPERIMENTAL

Soil Profile Analysis

Soil profiling was carried out to evaluate the physicochemical properties of soil supporting the growth of selected *Bougainvillea* cultivars under pot-cultivation conditions. Soil samples were collected from the rhizosphere region of established plants using a clean auger, air-dried at room temperature, and sieved to remove stones and debris prior to analysis.[10]

Plant Collection and Establishment

Bougainvillea spectabilis 'Torch Glow', *Bougainvillea* × *buttiana* 'Chitra', *Bougainvillea peruviana* 'Vishaka', and *Bougainvillea* × *buttiana* 'Mahara' 10-inch plant cuttings were procured from the Indian Council of Agricultural Research (ICAR)-Indian Agricultural Research Institute (IARI), New Delhi, and were brought to the Department of Botany, School of Bioengineering and Biosciences, LPU, Phagwara, Punjab. The well-prepared cuttings were planted into pots. To increase proper drainage and aeration, the base potting media, comprising soil, vermicompost and sand in a 40:30:30 (v/v) ratio, was amended with coco peat and perlite. Vermicompost and sand improved nutrient content and soil structure, while soil gave stability and support. The mixture was further enhanced with farmyard manure (FYM). Following transplantation, each pot weighed roughly 40 kg. To promote plant growth, a fertiliser mixture of water, urea, and DAP was administered. Regular irrigation was maintained, with watering occurring daily or twice a week, depending on environmental circumstances, providing roughly 5-10 litres of water per pot each time. To encourage healthy growth and development, Di-Ammonium Phosphate (DAP) fertiliser was used as needed, with additional applications indicated if plant growth appeared inadequate. A heavy DAP application was followed by water augmentation to guarantee optimal nutrient absorption. This management method guaranteed optimum aeration, nutrition, moisture availability, and overall plant health during the cultivation period. The pots were kept in a semi-shady area with a temperature range of 30- 35°C, and growth was observed for 3 months.[11]

Collection of *Bougainvillea* Leaves

The leaves of the four different *Bougainvillea* species, *Bougainvillea spectabilis* 'Torch Glow', *Bougainvillea* × *buttiana* 'Chitra', *Bougainvillea peruviana* 'Vishaka', and *Bougainvillea* × *buttiana* 'Mahara', were collected after 3 months and thoroughly washed and cleaned with distilled water to remove any surface contaminants and air-dried in a shaded area to retain their phytochemical integrity. After the drying process, the dry leaves were finally ground into uniform powder for further extraction procedures.[11]

Preparation of Leaf Extracts

To extract phytochemicals from the *Bougainvillea spectabilis* 'Torch Glow', *Bougainvillea* × *buttiana* 'Chitra', *Bougainvillea peruviana* 'Vishaka', and *Bougainvillea* × *buttiana* 'Mahara', a standardised solvent extraction method was followed. The prepared leaf powdered material was subjected to Soxhlet extraction using different solvents, such as methanol, ethanol, and hexane, to ensure the comprehensive extraction of bioactive compounds. To maximise the yield, the extraction process took a total of 6- 8 hours. The solvent extracts collected by rotary evaporator were concentrated under reduced pressure and stored in airtight containers at 4°C until further analysis. A total of 12 extracts were subsequently subjected to GC-MS (gas chromatography-mass spectrometry) analysis to compare and identify their phytochemical compositions.[12]

Gas Chromatography Mass Spectrometry (GC-MS) Analysis

The phytochemical constituents present in the leaf extracts of four *Bougainvillea* cultivars were characterised using gas chromatography–mass spectrometry (GC–MS) at the Central Instrumentation Facility (CIF), Lovely Professional University, Phagwara, Punjab, India. Analyses of methanolic, ethanolic, and hexane extracts were performed using a Shimadzu GC–MS QP-2010 system (Tokyo, Japan) fitted with an Rxi-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; 5% diphenyl/95% dimethyl polysiloxane) and an AOC-20i+s autosampler. Samples (2 μL) were injected into the system, with helium employed as the carrier gas. Mass spectra were acquired under electron ionisation (EI) mode at 70 eV. The column flow rate was maintained at 1.21 mL/min, while the total flow rate was 16.3 mL/min, corresponding to a linear velocity of 39.9 cm/s. The oven temperature program was initiated at 50°C, followed by heating to 250°C and holding for 5 min, then increased to 280°C over 22 min and held at this temperature until the end of the run. Data acquisition was carried out in ACQ mode over a scan range of m/z 40–700, using a scan interval of 0.50 s and an interface temperature of 260°C. The total analytical run time was 65 min. Individual constituents were tentatively identified by comparing their mass spectra with the NIST mass spectral library. Compounds whose best library match was a synthetic drug unlikely to occur in plant tissue were excluded.[13] Results are reported as presence/absence of each compound. The binary presence/absence matrix was used to construct the heat map and to perform hierarchical cluster analysis (HCA) based on Jaccard distance with average linkage (UPGMA), using Python (SciPy and Matplotlib libraries).

RESULTS AND DISCUSSION

Soil Profile Analysis

The soil had a strongly acidic pH (5.25) and normal electrical conductivity (142.8 μS/cm), indicating a non-saline soil. The level of organic carbon (0.422%) was low, while nitrogen (301.056 kg/ha) was medium, indicating moderate fertility. Phosphorus (3.2 ppm) and potassium (2.8 ppm) were low, which means that there might be a deficiency in nutrients (as indicated in Table-1). All in all, the soil is a moderately productive and slightly stressed environment on which *Bougainvillea* can grow. The pH of the soil is important in nutrient availability, and acidic soils may decrease the absorption of necessary nutrients, especially phosphorus. The normal EC indicates that soluble salts are not at a level that would cause osmotic stress. Organic carbon promotes microbial activity and nutrient cycling, whereas sufficient nitrogen encourages vegetative growth. Nevertheless, plants can have their enzymatic activity, stomatal regulation, and stress resistance curtailed by low potassium. These nutrient imbalances have been noted to affect the metabolism of plants as well as potentially changing the pattern of phytochemical accumulation, a phenomenon that affects phyto-transformations of plants in the future. In general, these conditions can cause mild abiotic stress, a response to which can be adaptive in plants, frequently resulting in increased

production of secondary metabolites with protective effects.[14] Similar observations have been reported in medicinal plants, where moderate abiotic stress enhances the accumulation of phenolics, flavonoids, and other secondary metabolites that contribute to plant defence and biological activity.[14]

Table-1: Soil Profile used for *Bougainvillea* Plant Species

S. No.	Soil test	Result	Interpretation
1	pH	5.25	Strongly acidic
2	Electrical Conductivity (EC)	142.8 µS/cm	Normal (non-saline)
3	Organic Carbon (OC)	0.422 %	Low
4	Nitrogen (N)	301.056 kg/ha	Medium
5	Phosphorus (P)	3.2 ppm	Low
6	Potassium (K)	2.8 ppm	Low

GC-MS Analysis

The comparative GC–MS profiling of leaf extracts from *Bougainvillea* varieties (Vishaka, Chitra, Mahara, and Torchglow) across different solvents revealed distinct solvent-dependent phytochemical patterns (Table-2; Fig.-1–12). Ethanol extracts showed a relatively uniform composition, with triethyl citrate consistently detected in all varieties, suggesting limited extraction diversity under this solvent system. In contrast, methanolic extracts exhibited the highest diversity of phytochemicals, including phenolic compounds, fatty acids (hexadecanoic acid, octadecanoic acid), phytol, and various bioactive derivatives. This aligns with previous findings that methanol efficiently extracts polar and semi-polar metabolites such as phenolics, contributing to higher phytochemical richness.[1,7] This may be attributed to the higher polarity of methanol, which facilitates the extraction of a broader range of polar and semi-polar metabolites, including phenolics and fatty acids. Flavonoids were not detected, as they are poorly amenable to GC–MS without prior derivatisation. Key compounds such as 2,4-di-tert-butylphenol, phytol, and hexadecanoic acid were consistently detected across all varieties, indicating their conserved presence and potential biological relevance. These compounds are widely reported to possess antioxidant, antimicrobial, and anti-inflammatory activities.[6] Hexane extracts predominantly contained non-polar compounds, including hydrocarbons (dotriacontane, tetrapentacontane), fatty acid derivatives, and siloxanes, highlighting the solvent's selectivity toward lipophilic constituents. Compounds such as neophytadiene, benzoic acid esters, and erucamide were widely distributed across varieties, supporting their role as common non-polar metabolites. Similar solvent-dependent partitioning of phytochemicals has been reported in GC–MS-based plant studies, where non-polar solvents preferentially isolate hydrocarbons and long-chain fatty compounds.[8] Varietal comparison indicated that Mahara exhibited the highest phytochemical diversity, followed by Vishaka and Torchglow, while Chitra showed comparatively fewer unique compounds.

Table-2: Compounds Identified Through GC-MS in *Bougainvillea* Varieties using different Solvents

S. No.	Compound Name	Extract Type	*Bougainvillea* varieties			
			Vishaka	Chitra	Mahara	Torchglow
1	Triethyl citrate	Ethanolic	✓	✓	✓	✓
2	4-Octanol, 2,4-dimethyl-	Methanolic	✓	✓	✓	✓
3	Pentanoic acid, 2-methyl-4-oxo-	Methanolic	✓	—	✓	✓
4	Phenol, dimethyl derivatives	Methanolic	✓	—	—	✓
5	Nonanoic acid	Methanolic	✓	—	—	✓
6	1,3-Dioxolane / 1,3-Dioxane derivatives	Methanolic	—	✓	✓	✓
7	2,4-Di-tert-butylphenol	Methanolic	✓	✓	✓	✓
8	D-Fructose, 3-O-methyl-	Methanolic	✓	✓	✓	✓
9	Adipamide	Methanolic	✓	✓	✓	✓
10	Tetradecanoic acid (Myristic acid)	Methanolic	✓	—	✓	—
11	Hexadecanoic acid/derivatives	Methanolic	✓	✓	✓	✓

12	Octadecanoic acid/glyceryl esters	Methanolic	✓	✓	✓	✓
13	Phthalic acid esters (various)	Methanolic	✓	✓	✓	✓
14	Phytol	Methanolic	✓	✓	✓	✓
15	Cyclohexasiloxane, dodecamethyl-	Hexane	—	✓	✓	—
16	Cycloheptasiloxane, tetradecamethyl-	Hexane	—	—	✓	—
17	Benzoic acid, 4-ethoxy-, ethyl ester	Hexane	✓	✓	✓	✓
18	Neophytadiene	Hexane	✓	✓	✓	✓
19	9-Octadecenitrile (Z-) / Oleonitrile	Hexane	✓	—	✓	—
20	13-Docosenamide (Z-) / Erucamide	Hexane	—	✓	✓	✓
21	Dotriacontane	Hexane	✓	✓	✓	✓
22	Tetrapentacontane	Hexane	✓	—	—	—
23	Oxirane derivative (Oxirane, 2,2-dimethyl-…)	Hexane	—	✓	—	—

✓ = Detected — = Not detected

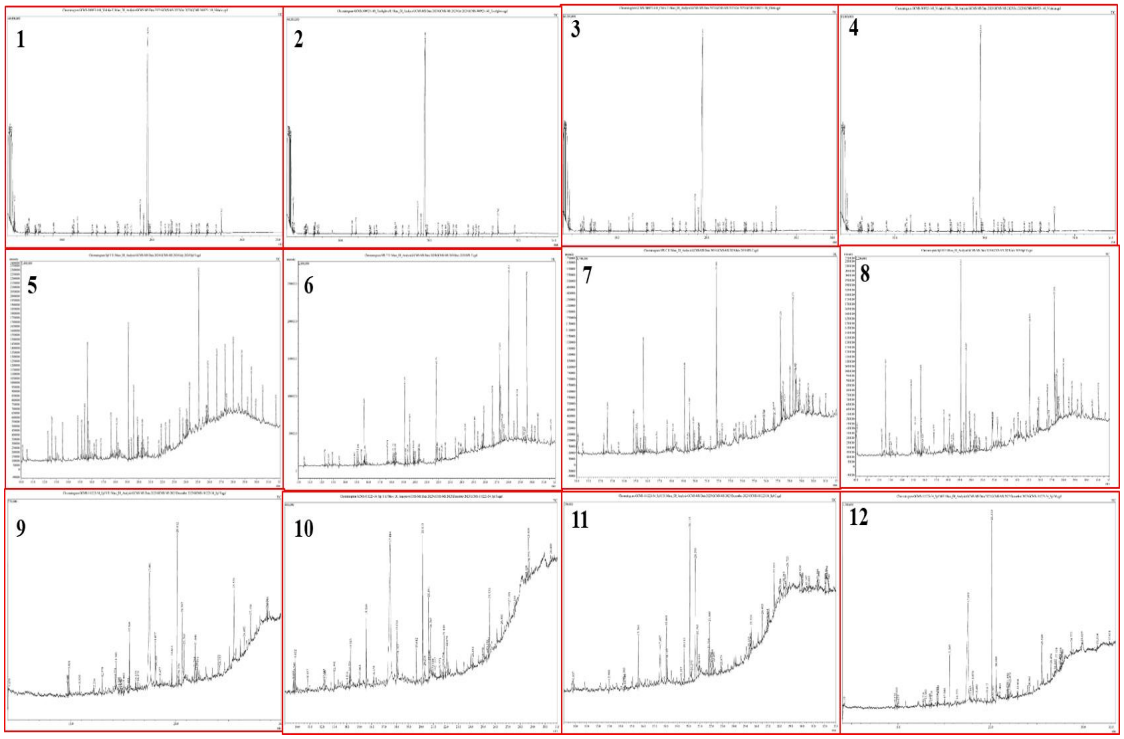


Fig.-1to 12: Representative GC–MS Chromatograms of Ethanolic, Hexane, and Methanolic Leaf Extracts from Four *Bougainvillea* Cultivars Showing Solvent-Dependent Phytochemical Profiles

The heat map analysis further supports these observations, demonstrating clustering patterns driven primarily by solvent polarity rather than varietal differences. This suggests that the extraction method plays a more dominant role than genetic variation in determining the detectable phytochemical profile under the studied conditions. Interestingly, several compounds identified in this study, including phytol, hexadecanoic acid, and benzoic acid derivatives, have been previously reported in *Bougainvillea* species and are associated with notable biological activities.[6] The presence of these compounds across multiple varieties reinforces the potential of *Bougainvillea* as a source of bioactive metabolites. Overall, the results

highlight that methanol is the most effective solvent for comprehensive phytochemical extraction, while hexane complements it by revealing non-polar constituents, together providing a more complete metabolic profile of *Bougainvillea* leaf extracts. Some of the detected compounds (e.g., triethyl citrate, phthalic acid esters, siloxanes, erucamide and 2,4-di-tert-butylphenol) are also reported as common laboratory or plasticiser-related contaminants; therefore, their plant origin should be interpreted with caution and confirmed using procedural blanks in future studies.

Multivariate Analysis of Phytochemical Profiles

Fig.-13 presents a heat map that shows the presence/absence of compounds that were identified by GC-MS in *Bougainvillea* varieties under various solvent systems. There are several compounds, such as phytol, hexadecanoic acid derivatives and 2,4-di-tert-butylphenol compounds, that were detected in all samples, hence a conserved core phytochemical profile. Interestingly, the chemical diversity of the methanolic extract was higher, which corroborates its application in the extraction of polar and semi-polar metabolites. Conversely, non-polar compounds like long-chain hydrocarbons and siloxanes dominated the hexane extracts, indicating a selectivity of the solvent. These results are in agreement with earlier reports that have demonstrated that the ability of solvents to extract phytochemicals is highly dependent on the polarity of the solvent.[1]

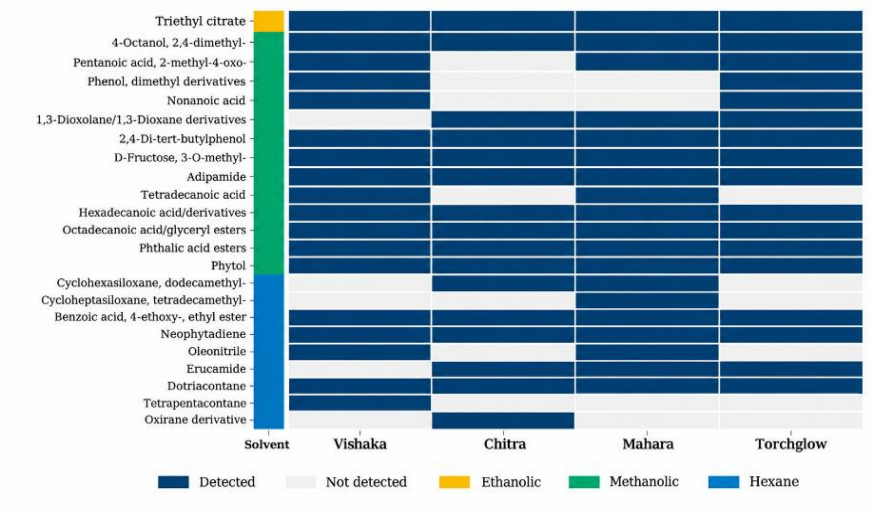


Fig.-13: Heat Map of the Presence–Absence Pattern of the 23 GC–MS-Identified Compounds Across Four *Bougainvillea* Cultivars. The Left Colour Strip Indicates the Extraction Solvent (Ethanolic, Methanolic or Hexane) under which Each Compound was Detected. Several Compounds Show Uniform Distribution Across all Varieties, Indicating a Shared Core Phytochemical Profile. Methanolic Extracts Display Higher Chemical Diversity, Reflecting Efficient Extraction of Polar Metabolites. Hexane Extracts Predominantly Reveal Non-Polar Compounds Such as Alkanes and Siloxanes.

Jaccard distance-based hierarchical cluster analysis (HCA) (Fig.-14) showed apparent clustering of samples largely based on extraction solvent instead of plant variety. Methanolic extracts clustered together, which means that they were similar in the composition of polar metabolites, whereas hexane extracts were in a separate cluster with non-polar phytochemicals. Ethanolic extracts were identical in composition (Jaccard distance = 0), as each contained only triethyl citrate. Within the methanolic extracts, Vishaka and Torchglow were the most similar (Jaccard distance = 0.154), whereas within the hexane extracts, Chitra and Torchglow were the most similar (0.333). These clustering patterns also reinforce the idea that solvent type is the most significant determinant of phytochemical differentiation that is frequently reported in GC-MS-based metabolomic investigations.[8] However, because each compound in Table-2 was recorded under a single extraction solvent, separation by solvent is partly inherent to the data structure; the HCA is therefore most informative for comparing cultivars within each solvent.

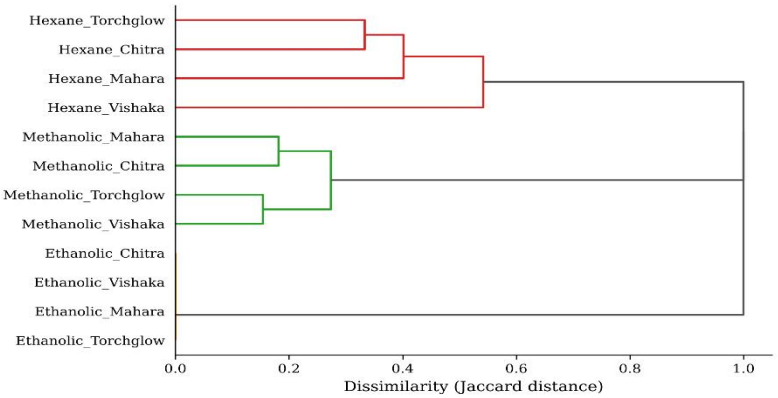


Fig.-14: Hierarchical Cluster Analysis (HCA) Dendrogram (Average Linkage, UPGMA) Based on Jaccard Distance Showing the Similarity Among GC–MS Phytochemical Profiles of *Bougainvillea* Leaf Extracts. The Clustering Reveals Clear Grouping of Samples According to Solvent Type, Highlighting Differences in Metabolite Composition among Methanolic, Hexane, and Ethanolic Extracts.

CONCLUSION

The solvent extracts analysed by GC-MS show that the phytochemical profile of the *Bougainvillea* leaf samples is highly dependent on the solvent used to extract them. Ethanolic extracts showed only triethyl citrate, a compound that may also arise from plasticiser contamination, whereas hexane extracts, especially in Mahara, contained a wider array of non-polar compounds, such as the diterpene neophytadiene, dotriacontane and aromatic esters. The presence of compounds like phytol and neophytadiene is consistent, indicating possible antioxidant, antibacterial, and anti-inflammatory activity. In general, the results indicate that hexane is an effective solvent to profile non-polar phytochemicals, and methanol increases the diversity of the total compound population. Plant-derived metabolites are well characterised by GC-MS, which is a reliable method of qualitative characterisation and indicates the pharmacognostic potential of the *Bougainvillea* species. Future studies should focus on the isolation, quantitative estimation, and biological validation of the identified bioactive compounds to further explore their pharmaceutical potential. This study provides a valuable foundation for the future utilisation of *Bougainvillea* species as a promising source of plant-derived bioactive compounds.

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CONFLICT OF INTERESTS

All authors have declared that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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