(ISSN: 0973-7049(P))

The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 21(4): S.I (4),771-801, 2026 www.thebioscan.com

Qualitative and quantitative screening of phytochemical compounds of *cymbidium bicolor* leaf extract and evaluation of biological activity

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Cite this paper as: Rashi. R and Dr Dwarakanath. V, Preliminary Phytochemical Profiling of Leaf Extracts of *Cymbidium bicolor*. *Corresponding author:* *dwarakanath@tumkuruniversity.ac.in*

DOI: https://doi.org/10.63001/tbs.2026.v21.i04.S.I(4).pp771-801

KEYWORDS

Cymbidium bicolor, phytochemical profiling, polar and non-polar solvents, total phenolic content, total flavonoid content, total alkaloid content, GC–MS.

Article Info

Received: 30/08 /2026

Accepted :18 /09/2026

Published: 28/10/2026

Abstract

"The medicinal plants are main sources of bioactive compounds with diverse pharmacological properties, in which cymbidium bicolor attracted scientific interest for the its rich phytochemical profile and therapeutic potentials. In this study, objective is to conduct a preliminary phytochemical profiling of cymbidium bicolor leaf extract, focusing on comparative solvent efficiency, quantification of key secondary metabolites, and also characterisation of the associated bioactive compounds. The shade dried leaf powder was experienced to Soxhlet extraction using solvents of different polarity, from non-polar to polar, to draw their comparative extraction efficiency. The Qualitative screening shows flavonoids, terpenoids, tannins, phenols, saponins, steroids, glycosides, coumarins, and quinones, with polar solvents providing a broader range of recognizable phyto-constituents than the non-polar solvent. Quantitative result of total alkaloid, flavonoids, and phenolics contents is carried out using standard spectrophotometric methods, with phenolic and flavonoid content expressed as gallic acid equivalents (GAE) and quercetin equivalents (QE), respectively. Ethanol, being a polar, shows markedly more extraction efficiency than the non-polar solvent, with yielding significantly more phenolic, flavonoid, and alkaloid contents, related to its ability to solubilise polar and minimally polar phytoconstituents meagre extracted by non-polar media. The ethanolic extraction was further characterised using FTIR spectroscopy to find out functional groups, and GC–MS analysis to identify major phytochemical content. These findings founded Cymbidium bicolor as a main source of pharmacologically related to secondary metabolites and highlight the effect of solvent polarity on phytoconstituent yield and diversity, giving a foundation for future isolation and characterisation of bioactive compounds with pharmaceutical application".

1. INTRODUCTION

The India has a rich historical background of Ayurvedic and traditional homemade system which is based on medicinal herbs, dating back to the early stage of Indian ancient past and shows a well-established historical rout of bioactive molecules. Although these phytocompounds were generally used in traditional medicinal practices, its mechanisms of use, pharmacological activities, and systematic uses were not scientifically documented by earlier medicinal practices. The Himalayan portion of India is known for its diversity for its geography and also the medicinal plants of different varieties and played major role in the development of related knowledge system relating to the medicine. Currently, India's health and medicine industry notified 280 medicinal plant species, in which some 175 are considered as threatened or vulnerable plants. The Indian Himalayas region is home to more than 8,000 species of angiosperms, 44 species of gymnosperms, and 600 species of pteridophytes (Pandey et al., 2013)

Among these, there are generally 1,748 varieties of plants are recognised as documented medicinal plants, shows the region's significant potential for ethnopharmacology and bioprospecting. The Himalayan region plant includes nearly 25,000 species, shows a vast natural storehouse of bio-

active and therapeutically important source, in which only about 10% scientifically explored and documented. This region is considered as biodiversity hotspots which greatly contribute to this floral wealth, accounting for about 11% of the world's plant diversity, with many species being endemic. Among angiosperms, the group Orchidaceous stands out as one of the vast and most environmentally advanced groups, with over 25,000 types of verities across more than 800 genera internationally. The ecological and ornamental importance of this diversity is in verge of extinction in which, many plant species face threats due to habitat loss, climate change, and human activities, underscoring the urgent need for protection and pharmacological documentation and research (Joshi et al., 2016)

Cymbidium bicolor

The genus Cymbidium (Orchidaceae), created by Olof Swartz in 1799, gets its name from the Greek word cymbi, which means "boat," and shows the characteristic shape of the flower's lip. It consists approximately 50 different species spread across Southeast Asia, parts of China, Japan, Indonesia, India, and also Australia. In India, there are 26 species are identified, including two endemics, with 15 species documented from the Nagaland. The recent field survey conducted on plant diversity in Nagaland, identified a previously undocumented varieties in the region. Based on morphological study and literature review, the species was confirmed as *Cymbidium bicolor Lindl*., marking its first documented occurrence in Nagaland's forest flora. *Cymbidium bicolor* is a terrestrial orchid, which is 20–70 cm tall, with narrowly ovoid pseudobulbs (6–10 cm) enclosed by 4–5 fibrous sheaths. The plant is 5–7 long, stiff in nature, arching leaves (40–70 cm). The inflorescence is arching to pendulous, gives 10–15 flowers. Flowers are 2.5–4 cm wide, with pale yellow sepals and petals marked by brown-maroon stripes and a white or cream-coloured lip with a maroon-brown central stripe and a yellow base patch on its area. The curved column has winged tips, and the plant provides cylindrical-ellipsoid fruits measuring 4–5 cm in length in general.

2. MATERIALS AND METHODS

2.1 Collection and Processing of the Plant Samples.

New green leaves of *Cymbidium bicolor* were collected from different natural habitats in forests of Kodagu District, Karnataka state of India. The collected plant remains was thoroughly processed with sterile distilled water to remove adhering soil particles, objects and other extraneous material, followed by mild blotting to remove excess moisture in it. Later, the leaves were then shade-dried under ambient conditions to minimise photolytic and thermal degradation of bioactive constituents. After complete drying of collected leaves, then the leaves were cut into small pieces and mechanically ground to get a coarse powder. The powdered samples were shifted to clean, airtight containers and stored at 4 °C until further experimental processing.

2.2 Preparation of Plant Extracts.

The extraction process was conducted making use of 5 different polar solvents and one non-polar solvent in a Soxhlet extraction apparatus. The system based on a round-bottom collecting flask attached to a Soxhlet chamber, with a reflux condenser mounted at the top. Accurately weighed powdered leaf samples were put into the extraction chamber, and the all solvents were added to the collecting flask. The extraction was made out by heating the system below the boiling point of each solvent to make sure efficient extraction while preventing thermal degradation of bioactive constituents. After completion of the extraction cycles, the resulting crude extracts were concentrated by solvent evaporation. The dried remnants were then transferred to clean, airtight containers and stored at 4 °C until further analysis.

Sl.no	Parameters	Soxhlet Extraction

1	Sample Size (g)	25 gm
2	Extraction Solvents	Methanol, Ethanol, Water, Acetone and Chloroform
3	Solvent Volume	300 ml
4	Temperature	Methanol(60°C), Ethanol(75°C), Water(95°C), Acetone (65°C) and Chloroform(70°C)
5	Time	6 Cycles of 3 Hrs

Table 1: Conditions used in Soxhlet Extraction

2.3 Qualitative phytochemical analysis

Qualitative tests were performed by using fresh, powdered samples, following standard procedures to identify the constituents as described.

1. **Detection of flavonoids**

   Ferric chloride test: 1 ml of extract treated with a few drops of Ferric chloride solution produces a blackish colour, indicating the presence of flavonoids (Shaikh & Patil, 2020)

   Alkaline reagent test: 1 ml of extract is treated with a few drops of sodium hydroxide solution. The formation of an intense yellow colour, which turns colourless upon the addition of dilute acid, indicates the presence of flavonoids.

   Lead acetate test: 1ml of the extract is treated with a few drops of lead acetate solution. The formation of a yellow-coloured precipitate indicates the presence of flavonoids (Shaikh & Patil, 2020)

2. **Detection of terpenoids**

   Salkowski test: To 2 ml of the extract, 2 mL of chloroform was added, followed by the careful addition of 2 mL of concentrated sulfuric acid along the side of the test tube. The mixture was gently shaken and allowed to stand. The appearance of a reddish-brown colour at the interface indicated the presence of terpenoids (Dauda et al., 2020)

3. **Detection of steroids**

   Libermann-Burchard test: 1 ml of extract was treated with 1 ml of acetic anhydride, and 1 ml of concentrated sulfuric acid was added carefully along the sides of the test tube. The appearance of a blue-green colour indicates the presence of steroids (Sukumar et al., 2020)

4. **Detection of coumarins:** 1 ml of extract treated with 1 ml of 10% NaOH solution; the formation of a yellow colour indicates the presence of coumarins (Ghoshal et al., 2022)
5. **Detection of tannins**: 1 ml of extract was treated with 1 ml of potassium dichromate, and the formation of a precipitate indicated the presence of tannins (Bhawna et al., 2023)
6. **Detection of phenols:** Add a few drops of 10% FeCl3 to the plant extract; the appearance of blue or green colour indicates the presence of phenols (Dauda et al., 2020)
7. **Detection of saponins: Frothing test:** Add 0.5 ml of plant extract to 5 ml of distilled water and froth to check for the presence of saponins (Bhawna et al., 2023)
8. **Detection of anthocyanins:** A few ml of plant extract was treated with 10% NaOH, and the blue colour indicates the presence of anthocyanins (Godlewska et al., 2023)
9. **Detection of glycosides: Keller-Killiani Test:** Plant extract is treated with a few drops of glacial acetic acid and ferric chloride solution, then mixed. Concentrated sulphuric acid is added, and the mixture is observed for the formation of two layers. A lower reddish-brown layer and an upper acetic acid layer, which turns bluish-green, indicate a positive test for glycosides (Godlewska et al., 2023)

10. **Detection of quinones:** To 1ml of extract, 1ml of con.H2SO4 was added; formation of red colour indicates the presence of quinones (Dauda et al., 2020)
11. **Detection of alkaloids**
    Mayer's Test: 1ml of Extracts treated with a few drops of Mayer's reagent, resulting in the appearance of a white creamy precipitate, indicating the presence of Alkaloids.
    Hager's Test: 1ml of extracts treated with a few drops of Hager's reagent, resulting in the formation of a yellow precipitate, indicates the presence of alkaloids (Ghoshal et al., 2022)

3. GC-MS

The mass spectrum obtained from GC-MS analysis was interpreted using the National Institute of Standards and Technology (NIST) database, which contains over 62,000 reference patterns. The spectra of the unknown compounds were compared with those of known compounds in the NIST library, enabling identification of the names and structures of the components present in the test material (Simón-Manso et al., 2013)

3.1 Quantitative identification of bioactive compounds by GC-MS.

GC-MS analysis of these extracts was carried out using the GC 2030 TQ 8040NX SCHIMADZU Model with the following conditions.

Table2: GCMS Conditions

GC CONDITIONS	
Column oven temperature	100 °C
Injection volume	1 microliter
Injection Temperature	280 °C
Column flow rate	0.95 ml/min
Column length	30 mt
Column thickness	0.25mm
Pre-column pressure	68.3 KPa
Carrier gas	Helium

MS CONDITIONS	
Ionization mode	EI
Ion source temperature	250° C
Interface Temperature	280° C
Mass-to-charge ratio (m/z)	50 to 550

4. Determination of Total Alkaloid Content in Five Different Extracts by Harborne Gravimetric Method

The total alkaloid content was assessed using the gravimetric method outlined by Harborne. Dried plant extract was combined with 100 ml of 10% acetic acid in ethanol and allowed to sit for 4 hours at room temperature. The mixture was then filtered, concentrated to one-third of its initial volume using a water bath, and treated dropwise with concentrated ammonium

hydroxide until complete precipitation was observed. The resulting precipitate was permitted to settle, rinsed with dilute ammonium hydroxide, and gathered using pre-weighed Whatman No. 42 filter paper. The residue was dried until a constant weight was reached, and the total alkaloid content was calculated by determining the difference between the final and initial weights, expressed as g/100 g of dry sample (Van Tan, 2018b)

5. Determination of Total Flavonoid Content in Five Different Extracts by Aluminium Chloride Method

The total flavonoid content (TFC) of the *Cymbidium bicolor* extracts was quantified using the aluminium chloride colorimetric technique, in accordance with the method outlined by Chang et al. (2002), utilising quercetin as the reference standard. In summary, 0.5 ml of each extract was combined with 1.5 mL of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate, and 2.8 mL of distilled water. The mixture was left to react at room temperature for 30 minutes, and the absorbance was recorded at 415 nm using a UV–Visible spectrophotometer, compared to a reagent blank. A calibration curve for quercetin was employed to determine TFC, expressed in milligrams of quercetin equivalents per gram of dry extract (mg QE/g) (Nicolescu et al., 2025)

6. Determination of Total Phenolic Content in Five Different Extracts by Folin–Ciocalteu Method

The total phenolic content (TPC) of the extracts from *Cymbidium bicolor* was assessed through the Folin–Ciocalteu colorimetric method, using gallic acid as the standard reference. In summary, 0.2 ml of each plant extract or a standard solution of gallic acid (ranging from 20 to 100 µg/ml) was combined with 1 ml of 10% (v/v) Folin–Ciocalteu reagent and left to incubate at room temperature for 5 minutes. Next, 800 µl of a 7.5% (w/v) sodium carbonate solution was incorporated, and the mixture was allowed to incubate in a dark environment for 30 minutes. The absorbance was recorded at 765 nm using a UV–Visible spectrophotometer, with a reagent blank serving as the reference. The TPC was calculated based on the gallic acid calibration curve and presented as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g) (Lucas et al., 2022)

7. Determination of Antioxidant Activity by DPPH Assay

The antioxidant activity of *Cymbidium bicolor* leaf extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay. DPPH is a stable violet-coloured free radical that absorbs maximally at 517 nm and is reduced upon accepting an electron or hydrogen atom from antioxidant compounds, resulting in a decrease in absorbance. Ascorbic acid served as the reference standard at working concentrations of 10–100 µg/mL, while the extracts were tested at 25–800 µg/ml. The assay was performed in 96-well microplates by mixing 100 µl of DPPH solution with 100 µl of each standard or extract solution. The control contained 100 µl of DPPH solution and 100 µl of methanol, while the blank contained 200 µl of methanol. The reaction mixtures were incubated in the dark at room temperature for 30 min, and absorbance was measured at 517 nm using a microplate reader. Radical-scavenging activity was expressed as percentage inhibition using the formula: % inhibition = [(Ac − As)/Ac] × 100, where Ac represents the absorbance of the control and as represents the absorbance of the sample or standard (Baliyan et al., 2022)

8. Determination of Antioxidant Activity by ABTS Assay

The antioxidant activity of *Cymbidium bicolor* leaf extracts was evaluated using the ABTS [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] radical cation decolourisation assay. The ABTS radical cation (ABTS•+) was

generated by mixing equal volumes of 7 mM ABTS and 2.45 mM potassium persulfate solutions, followed by incubation in the dark at room temperature for 12–16 h. The resulting solution was diluted with methanol or phosphate buffer to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Trolox was used as the reference standard at concentrations of 20–100 µg/ml. For the assay, 200 µL of the diluted ABTS•+ solution was mixed with 10 µL of each standard or extract concentration in a 96-well microplate. Methanol was used in place of the sample for the control. The reaction mixtures were incubated at room temperature for 6 min, and absorbance was measured at 734 nm using a microplate reader. Antioxidant activity was expressed as percentage inhibition of the ABTS radical cation using the formula: % inhibition = [(Ac − As)/Ac] × 100, where Ac is the absorbance of the control and As is the absorbance of the sample or standard (Ozgen et al., 2006)

9. RESULTS

9. 1 Extraction Yield in Different Solvent Systems.

The extraction result serves as an indicator of the solvent's efficiency in isolating defined bioactive components from the plant material. It gives insight into the extractability of the plant under varying situations. The percentage yield was calculated based on the dry weight of the sample using the following formula:

% Yield of extract (g/100 g) = (W_1 × 100) / W_2

Where **W_1** = Weight of the extract residue after solvent evaporation and **W_2** = Weight of the dried plant powder used for extraction.

Name of the plant	Plant part used	Solvent used for extraction	% yield	Colour
Cymbidium bicolor	Leaves	Methanol	13.76%	Green
		Ethanol	5.28%	Dark green
		Water	10%	Brown
		Acetone	5.08%	Light brown
		Chloroform	11.53%	Dark green

Table 3: Results of Soxhlet extraction

The yield of extracts from different solvents depends on the composition of phytoconstituents and the polarity of the solvent. The basic parameters that influence the quality of an extract are the plant part used as the starting material, the solvent used for extraction, and the extraction procedure. Our results showed that the maximum percentage yield was obtained when the leaves of *Cymbidium bicolor* were extracted by Soxhlet extraction, yielding Methanol (13.76%), followed by Aqueous (10%), Ethanol (5.28%), and Acetone (5.08%). Our results showed that Methanol was more efficient in extracting phytochemicals than other solvents.

9. 2 Qualitative phytochemical analysis

Chemical test	Leaf extract				
	Methanolic	Ethanolic	Aqueous	Acetone	Chloroform
1. Test for Flavonoid					
Ferric chloride test	(+)	(+)	(+)	(+)	(+)
Alkaline Reagent test	(+)	(+)	(+)	(+)	(+)
Lead acetate test	(+)	(+)	(+)	(+)	(+)

2. Test for Terpenoids					
Salkowski test	(+)	(+)	(+)	(+)	(+)
3. Test for Alkaloids					
Hager's test	(-)	(-)	(-)	(-)	(-)
Mayer's test	(-)	(-)	(-)	(-)	(-)
4. Test for Tannins					
Braymer's test	(-)	(-)	(-)	(-)	(-)
10% NaOH test	(-)	(-)	(-)	(-)	(-)
Bromine water test	(-)	(-)	(-)	(-)	(-)
5. Test for Phenols					
Ferric chloride test	(+)	(+)	(+)	(+)	(+)
Ellagic Acid test	(+)	(+)	(+)	(+)	(+)
6. Test for Quinones					
Conc. HCl test	(-)	(-)	(-)	(-)	(-)
Sulphuric acid test	(-)	(-)	(-)	(-)	(-)
7. Test for Anthocyanins	(-)	(-)	(-)	(-)	(-)
8. Test for Glycosides	(-)	(-)	(-)	(-)	(-)
9.Test for Coumarins	(+)	(+)	(+)	(+)	(+)
10.Test for Saponins	(+)	(+)	(+)	(+)	(+)

Table 4: Results of Qualitative analysis of different extracts of leaf of *Cymbidium bicolor*

9.3 ETHANOLIC EXTRACT

The chemical constituents present in the ethanolic leaf extract of *Cymbidium bicolor* were identified using Gas Chromatography–Mass Spectrometry (GC–MS) analysis. The chromatogram of the ethanolic leaf extract of *Cymbidium bicolor* showed 60 peaks, which indicated the presence of 60 different bioactive compounds.

(ISSN: 0973-7049(P))

The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 21(4): S.I (4),771-801, 2026 www.thebioscan.com

GC-MS Chromatogram of Ethanolic Extract of the leaf of *Cymbidium bicolor*

Sample Information

Analyzed by	: THEJASWI
Analyzed	: 09/10/2025 16:54:09
Sample Type	: Unknown
Level #	: 1
Sample Name	: Ethanol
Sample ID	: Ethanol
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 5
Injection Volume	: 1.00
Data File	: D:\GCMS Scan\Ethanol_Ethanol_09-10-2025_5.qgd
Org Data File	: D:\GCMS Scan\Ethanol_Ethanol_09-10-2025_5.qgd
Method File	: D:\GCMS Scan\General SCAN method_18072023 - Copy.qgm
Org Method File	: D:\GCMS Scan\General SCAN method_18072023 - Copy.qgm
Report File	:
Tuning File	: D:\EHS DATA 2025\Tuning file\09102025.qgt
Modified by	: Admin
Modified	: 09/10/2025 18:34:44

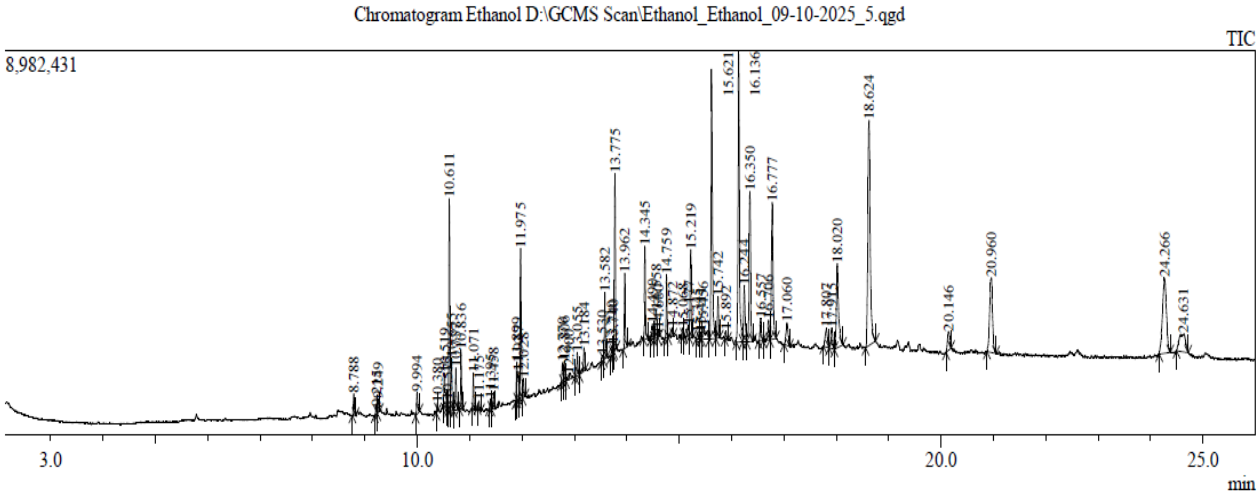


Figure 1: GC–MS chromatogram of the ethanolic leaf extract of *Cymbidium bicolor*, showing the separation and relative abundance of the detected compounds at different retention times

Peak #	Name of the compound	R.Time	Area %	Molecular Formula	Molecular weight
1	γ-Sitosterol	18.624	11.67	$C_{29}H_{50}O$	414.70
2	β-Tocopherol	16.136	9.35	$C_{28}H_{48}O_2$	416.68
3	δ-Tocopherol	15.621	8.05	$C_{27}H_{46}O_2$	402.65
4	Phytyl linoleate	24.266	6.57	$C_{38}H_{72}O_2$	561.00
5	Vitamin E	16.777	4.91	$C_{29}H_{50}O_2$	430.70
6	Hexatriacontane	16.350	4.82	$C_{36}H_{74}$	506.97
7	Phytyl stearate	20.960	4.77	$C_{38}H_{74}O_2$	563.00
8	Stigmasterol	18.020	4.28	$C_{28}H_{48}O$	412.70
9	Neophytadiene	10.611	3.93	$C_{20}H_{38}$	278.52

10	Bis(2-ethylhexyl) phthalate	13.775	3.18	$C_{24}H_{38}O_4$	390.56
11	Phytol	11.975	3.12	$C_{20}H_{40}O$	296.54
12	Phytyl eicosanoate	24.631	2.51	$C_{40}H_{78}O_2$	591.00
13	Hexatriacontane	15.219	2.06	$C_{36}H_{74}$	506.97
14	γ-Tocopherol	16.244	2.00	$C_{28}H_{48}O_2$	416.68
15	Tetratetracontane	14.345	1.99	$C_{44}H_{90}$	619.20
16	Tetratetracontane	13.962	1.60	$C_{44}H_{90}$	619.20
17	Tetratetracontane	13.582	1.48	$C_{44}H_{90}$	619.20
18	2-Pentadecanone, 6,10,14-trimethyl-	10.655	1.29	$C_{18}H_{36}O$	268.48
19	Tetracontane	14.759	1.29	$C_{40}H_{82}$	563.09
20	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	10.737	1.07	$C_{20}H_{40}O$	296.53

Table 5: The active principles, along with their retention times (RT), peak area concentrations (%), molecular formulas, and molecular weights, are presented in the Table.

	Type of the constituents		
Area % values	Major constituents (more than 10%)	Minor constituents (between 1-10%)	Trace constituents (less than 1%)
No.of Compounds identified	01	19	-

Table 6: Among the identified bioactive components, γ-sitosterol (11.67%), β-tocopherol (9.35%), and δ-tocopherol (8.05%) exhibited the highest percentage peak areas, followed by phytyl linoleate (6.57%), vitamin E (4.91%), and stigmasterol (4.28%).

9.4 METHANOLIC EXTRACT

The chemical constituents present in the methanolic leaf extract of *Cymbidium bicolor* were identified using Gas Chromatography–Mass Spectrometry (GC–MS) analysis. The chromatogram of the methanolic leaf extract of *Cymbidium bicolor* showed 72 peaks, which indicated the presence of 72 different bioactive compounds.

GC-MS Chromatogram of methanolic extract of leaf of *Cymbidium bicolor*

(ISSN: 0973-7049(P))

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Sample Information

Analyzed by	: THEJASWI
Analyzed	: 09/10/2025 15:19:45
Sample Type	: Unknown
Level #	: 1
Sample Name	: Methanol
Sample ID	: Methanol
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 2
Injection Volume	: 1.00
Data File	: D:\GCMS Scan\Methanol_Methanol_09-10-2025_2.qgd
Org Data File	: D:\GCMS Scan\Methanol_Methanol_09-10-2025_2.qgd
Method File	: D:\GCMS Scan\General SCAN method_18072023 - Copy.qgm
Org Method File	: D:\GCMS Scan\General SCAN method_18072023 - Copy.qgm
Report File	:
Tuning File	: D:\EHS DATA 2025\Tuning file\09102025.qgt
Modified by	: Admin
Modified	: 09/10/2025 18:38:54

Chromatogram Methanol D:\GCMS Scan\Methanol_Methanol_09-10-2025_2.qgd

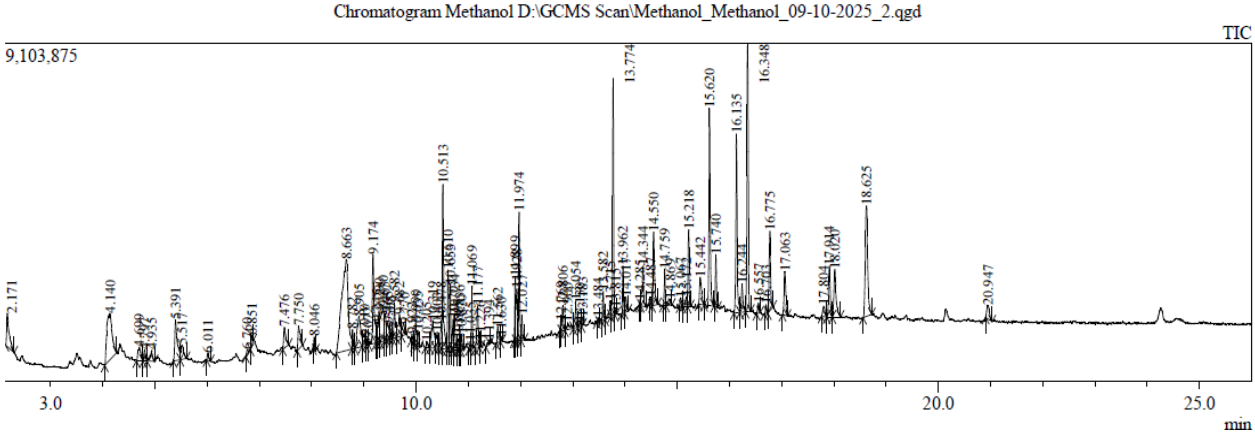


Figure 2: GC–MS chromatogram of the methanolic leaf extract of *Cymbidium bicolor*, showing the separation and relative abundance of the detected compounds at different retention times.

Peak#	Name of the compound	R.Time	Area %	Molecular Formula	Molecular weight
1	2-(Isobutoxymethyl)oxirane	8.663	10.58	$C_7H_{14}O_2$	130.18
2	Glycerin	4.140	4.44	$C_3H_8O_3$	92.09
3	Bis(2-ethylhexyl) phthalate	13.774	3.82	$C_{24}H_{38}O_4$	390.56
4	(S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3-oxobut-1…)	10.513	3.48	$C_{13}H_{18}O_3$	222.28
5	Phenol, 4-ethenyl-2,6-dimethoxy-	9.174	2.74	$C_{10}H_{12}O_3$	180.20
6	Phytol	11.974	2.19	$C_{20}H_{40}O$	296.54
7	Phenol, 2-methoxy-	5.391	1.88	$C_7H_8O_2$	124.14
8	β-D-Glucopyranose, 1,6-anhydro-	8.905	1.85	$C_6H_{10}O_5$	162.14
9	L-Arabinitol	9.370	1.83	$C_5H_{12}O_5$	152.15
10	4-(4-Hydroxy-3-methoxystyryl)-2,6-dimethoxy…	14.550	1.75	$C_{17}H_{18}O_5$	302.33
11	Propanoic acid, 2-oxo-, methyl ester	2.171	1.50	$C_6H_4O_3$	102.09
12	Loliolide	10.319	1.29	$C_{11}H_{16}O_3$	196.24
13	2-Pentadecanone, 6,10,14-trimethyl-	10.653	1.20	$C_{18}H_{36}O$	268.48
14	Neophytadiene	10.610	1.19	$C_{14}H_{18}O$	222.28
15	9,12,15-Octadecatrienoic acid, methyl ester, (Z…)	11.928	1.07	$C_{19}H_{34}O_2$	294.47

16	Octadecanoic acid, phenylmethyl ester	9.682	1.02	$C_6H_{12}O_6$	180.16
17	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	11.899	1.02	$C_{19}H_{34}O_2$	294.47
18	Hexadecanoic acid, methyl ester	11.069	0.93	$C_{17}H_{34}O_2$	270.45
19	Phenol, 2,6-dimethoxy-	7.750	0.91	$C_8H_{10}O_3$	154.16
20	Megastigmatrienone	9.582	0.91	$C_{25}H_{42}O_2$	374.60

Table 7: The active principles with their retention time (RT), concentration of peak area (%), molecular formula and molecular weight are presented in the table.

	Type of the constituents		
Area % values	Major constituents (more than 10%)	Minor constituents (between 1-10%)	Trace constituents (less than 1%)
No. Of Compounds identified	01	16	03

Table 8: Among the identified bioactive components, 2-(isobutoxymethyl) oxirane (10.58%), glycerin (4.44%), and bis(2-ethylhexyl) phthalate (3.82%) exhibited the highest percentage peak areas, followed by (S, E)-4-hydroxy-3,5,5-trimethyl-4-(3-oxobut-1…) (3.48%), phenol, 4-ethenyl-2,6-dimethoxy- (2.74%), and phytol (2.19%).

9. 5 AQUEOUS EXTRACT

The chemical constituents present in the aqueous leaf extract of *Cymbidium bicolor* were identified using Gas Chromatography–Mass Spectrometry (GC–MS) analysis. The chromatogram of the Aqueous leaf extract of *Cymbidium bicolor* showed 74 peaks, which indicated the presence of 74 different bioactive compounds.

GC-MS Chromatogram of aqueous extract of the leaf of *Cymbidium bicolor*

Sample Information

Analyzed by	: THEJASWI
Analyzed	: 09/10/2025 16:22:40
Sample Type	: Unknown
Level #	: 1
Sample Name	: Aqueous
Sample ID	: Aqueous
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 4
Injection Volume	: 1.00
Data File	: D:\GCMS Scan\Aqueous_Aqueous_09-10-2025_4.qgd
Org Data File	: D:\GCMS Scan\Ethyl Acetate_Ethyl Acetate_09-10-2025_4.qgd
Method File	: D:\GCMS Scan\General SCAN method_18072023 - Copy.qgm
Org Method File	: D:\GCMS Scan\General SCAN method_18072023 - Copy.qgm
Report File	:
Tuning File	: D:\EHS DATA 2025\Tuning file\09102025.qgt
Modified by	: Admin
Modified	: 09/10/2025 18:37:07

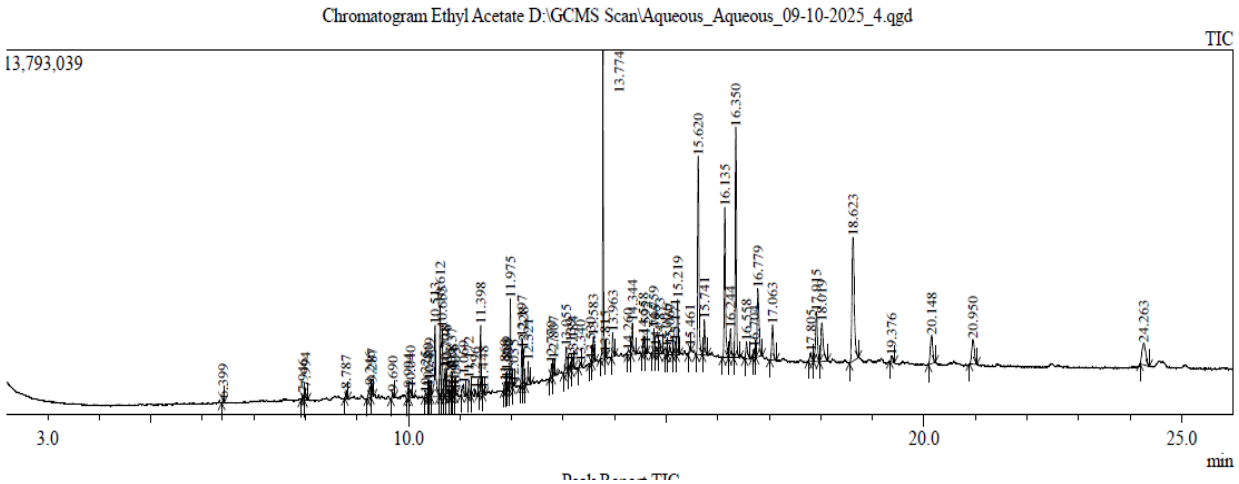


Figure 4: GC–MS chromatogram of the aqueous leaf extract of *Cymbidium bicolor*, showing the separation and relative abundance of the detected compounds at different retention times.

Peak #	Name of the compound	R.Time	Area %	Molecular Formula	Molecular weight
1	Hexatriacontane	16.350	9.34	$C_{36}H_{74}$	506.97
2	γ-Sitosterol	18.623	8.85	$C_{29}H_{50}O$	414.70
3	Bis(2-ethylhexyl) phthalate	13.774	7.79	$C_{24}H_{38}O_4$	390.56
4	δ-Tocopherol	15.620	7.78	$C_{27}H_{46}O_2$	402.65
5	β-Tocopherol	16.135	6.45	$C_{28}H_{48}O_2$	416.68
6	(S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-…)*	10.513	3.64	$C_{13}H_{18}O_3$	222.28
7	Vitamin E	16.779	3.98	$C_{29}H_{50}O_2$	430.71
8	Stigmasterol	18.019	2.75	$C_{29}H_{48}O$	412.69
9	Neophytadiene	10.612	2.42	$C_{20}H_{38}$	278.52
10	Phytyl linoleate	24.263	2.49	$C_{36}H_{64}O_2$	544.90
11	Phytol	11.975	2.55	$C_{20}H_{40}O$	296.54
12	Stigmast-4-en-3-one	20.148	2.04	$C_{29}H_{48}O$	412.70
13	2-Pentadecanone, 6,10,14-trimethyl-	10.655	1.98	$C_{18}H_{36}O$	268.48
14	Phytyl stearate	20.950	1.96	$C_{38}H_{74}O_2$	563.00

15	Hexatriacontane	15.219	1.83	$C_{36}H_{74}$	506.97
16	γ-Tocopherol	16.244	1.48	$C_{28}H_{48}O_2$	416.69
17	Linoleic acid ethyl ester	12.197	1.33	$C_{20}H_{36}O_2$	308.50
18	1,8,11,14-Heptadecatetraene, (Z,Z,Z)-	12.228	1.26	$C_{17}H_{28}$	232.40
19	Hexatriacontane	14.344	1.17	$C_{36}H_{74}$	506.98
20	4,8,12,16-Tetramethylheptadecan-4-olide	13.055	1.01	$C_{21}H_{40}O_2$	324.54

Table 9: The active principles, along with their retention times (RT), peak area concentrations (%), molecular formulas, and molecular weights, are presented in the Table.

	Type of the constituents		
Area % values	Major constituents (more than 10%)	Minor constituents (between 1-10%)	Trace constituents (less than 1%)
No. of Compounds identified	-	17	03

Table 10: Among the identified bioactive compounds, hexatriacontane (9.34%), γ-sitosterol (8.85%), bis(2-ethylhexyl) phthalate (7.79%), and δ-tocopherol (7.78%) exhibited the highest percentage peak areas, followed by β-tocopherol (6.45%) and vitamin E (3.98%).

9. 6 ACETONE EXTRACT

The chemical constituents present in the acetone leaf extract of *Cymbidium bicolor* were identified using Gas Chromatography–Mass Spectrometry (GC–MS) analysis. The chromatogram of the Acetone leaf extract of *Cymbidium bicolor* showed 72peaks, which indicated the presence of 74 different bioactive compounds.

GC-MS Chromatogram of the acetone extract of the leaf of *Cymbidium bicolor*

(ISSN: 0973-7049(P))

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Sample Information

Analyzed by	: THEJASWI
Analyzed	: 09/10/2025 15:51:10
Sample Type	: Unknown
Level #	: 1
Sample Name	: Acetone
Sample ID	: Acetone
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 3
Injection Volume	: 1.00
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Report File	:
Tuning File	: D:\EHS DATA 2025\Tuning file\09102025.qgt
Modified by	: Admin
Modified	: 09/10/2025 18:36:02

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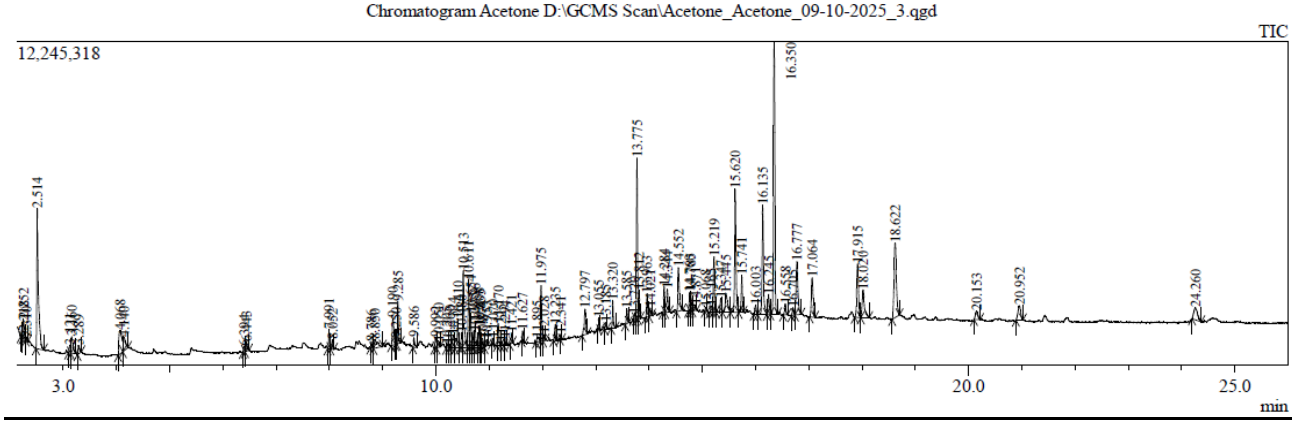


Figure 5: GC–MS chromatogram of the acetone leaf extract of *Cymbidium bicolor*, showing the separation and relative abundance of the detected compounds at different retention times.

Peak#	Name of the compound	R.Time	Area %	Molecular Formula	Molecular weight
1	2-Pentanone, 4-hydroxy-4-methyl-	2.514	6.82	$C_6H_{12}O_2$	116.16
2	δ-Tocopherol	15.620	5.30	$C_{27}H_{46}O_2$	402.65
3	β-Tocopherol	16.135	4.96	$C_{28}H_{48}O_2$	416.68
4	Bis(2-ethylhexyl) phthalate	13.775	4.32	$C_{24}H_{38}O_4$	390.56
5	(S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-…)	10.513	3.37	$C_{13}H_{18}O_3$	222.28
6	Glycerin	4.068	2.13	$C_3H_8O_3$	92.09
7	Neophytadiene	10.611	2.01	$C_{20}H_{38}$	278.52
8	4-(4-Hydroxy-3-methoxystyryl)-2,6-dimethoxy…	14.552	2.00	$C_{17}H_{18}O_5$	302.33
9	Tetratetracontane	15.219	1.81	$C_{44}H_{90}$	619.19
10	Phytol	11.975	1.69	$C_{20}H_{40}O$	296.54
11	Phenol, 4-ethenyl-2,6-dimethoxy-	9.190	1.50	$C_{10}H_{12}O_3$	180.20
12	Callosumin	15.445	1.45	$C_{16}H_{16}O_4$	272.29
13	Hexatriacontane	15.741	1.45	$C_{36}H_{74}$	506.99
14	Heptadecane	10.410	1.35	$C_{17}H_{36}$	240.48
15	Cyclodecasiloxane, eicosamethyl-	12.797	1.27	$C_{20}H_{60}O_{10}Si_{10}$	741.54
16	Tetradecane	9.285	1.25	$C_{14}H_{30}$	198.39
17	2-Pentadecanone, 6,10,14-trimethyl-	10.654	1.12	$C_{18}H_{36}O$	268.48

18	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	10.736	1.03	$C_{20}H_{40}O$	296.54
19	Hexatriacontane	14.344	1.02	$C_{36}H_{74}$	506.97
20	Phenol	4.140	1.01	C_6H_6O	94.11

Table 11: The active principles, along with their retention times (RT), peak area concentrations (%), molecular formulas, and molecular weights, are presented in the Table.

Area % values	Type of the constituents		
	Major constituents (more than 10%)	Minor constituents (between 1-10%)	Trace constituents (less than 1%)
No.of Compounds identified	-	20	-

Table 12: Among the identified bioactive components, 2-Pentanone, 4-hydroxy-4-methyl- (6.82%) has the highest per cent peak area.

9.7 CHLOROFORM EXTRACT

The chemical constituents present in the chloroform leaf extract of *Cymbidium bicolor* were identified using Gas Chromatography–Mass Spectrometry (GC–MS) analysis. The chromatogram of the Aqueous leaf extract of *Cymbidium bicolor* showed 55 peaks, which indicated the presence of 55 different bioactive compounds.

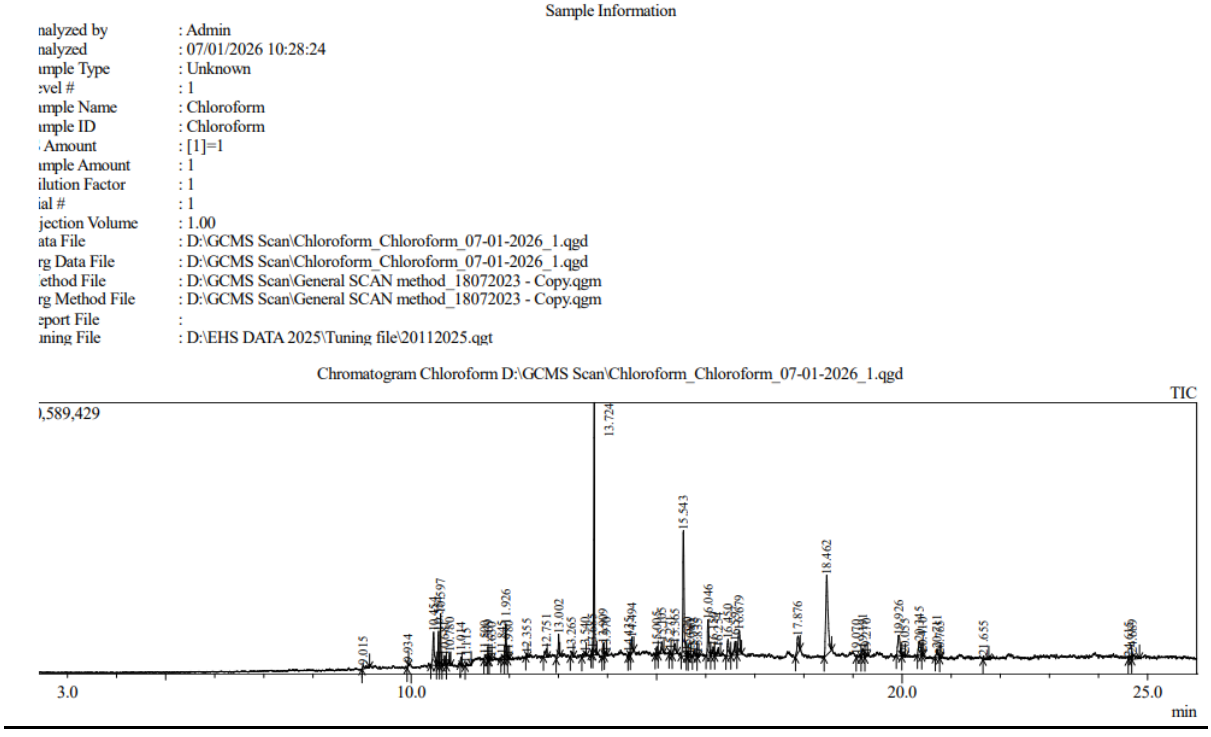


Figure 6: GC–MS chromatogram of the chloroform leaf extract of *Cymbidium bicolor*, showing the separation and relative abundance of the detected compounds at different retention times.

Peak #	Name of the compound	R.Time	Area %	Molecular Formula	Molecular weight
1	Bis(2-ethylhexyl) phthalate	13.724	15.30	$C_{24}H_{38}O_4$	390.56

2	β-Sitosterol	18.462	13.86	$C_{29}H_{50}O$	414.71
3	δ-Tocopherol	15.543	12.31	$C_{27}H_{46}O_2$	402.65
4	(R)-6-Methoxy-2,8-dimethyl-2-((4R,8R)-4,8,12-trimethyltridecyl)chroman	16.046	4.11	$C_{28}H_{48}O_2$	416.68
5	R-Limonene	10.454	3.88	$C_{10}H_{16}$	136.24
6	Stigmast-4-en-3-one	19.926	3.50	$C_{29}H_{48}O$	412.70
7	α-Tocopheryl acetate	16.679	3.25	$C_{31}H_{52}O_3$	472.74
8	2,15-Hexadecanedione*	10.597	3.13	$C_{16}H_{30}O_2$	254.41
9	Phytol	11.926	2.88	$C_{20}H_{40}O$	296.54
10	Stigmasterol	17.876	2.44	$C_{29}H_{48}O$	412.70
11	Stigmasta-3,5-dien-7-one	20.345	2.44	$C_{29}H_{46}O$	410.67
12	7-Dehydrodiosgenin	16.450	2.12	$C_{27}H_{40}O_2$	396.60
13	trans-11-Tetradecenyl acetate	10.554	1.95	$C_{16}H_{30}O_2$	254.41
14	Stigmasta-3,5-diene	16.597	1.72	$C_{29}H_{48}$	396.70
15	Hexanoic acid, 4-tetradecyl ester	13.002	1.72	$C_{20}H_{40}O_2$	312.53
16	2-Cyclopropene-1-carboxylic acid, 2-(2-naphthalenyl)-3-phenyl-, methyl ester	15.365	1.50	$C_{21}H_{16}O_2$	300.35
17	Dihydro-normorphine, 3-desoxy-	14.494	1.35	$C_{16}H_{19}NO_3$	273.33
18	Succinic acid, dodec-2-en-1-yl pentafluorophenyl ester	19.191	1.35	$C_{22}H_{27}F_5O_4$	450.44
19	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate	20.711	1.27	$C_{22}H_{42}O_2$	338.57
20	Tetracosamethyl-cyclododecasiloxane	24.689	1.24	$C_{24}H_{72}O_{12}Si_{12}$	889.85

Table 13: The active principles, along with their retention times (RT), peak area concentrations (%), molecular formulas, and molecular weights, are presented in the Table.

	Type of the constituents		
Area % values	Major constituents (more than 10%)	Minor constituents (between 1-10%)	Trace constituents (less than 1%)
No. of Compounds identified	03	17	-

Table 14: Among the identified compounds, bis(2-ethylhexyl) phthalate (15.30%), β-sitosterol (13.86%), and δ-tocopherol (12.31%) exhibited the highest percentage peak areas, followed by (R)-6-methoxy-2,8-dimethyl-2-((4R,8R)-4,8,12-trimethyltridecyl)chroman (4.11%), R-limonene (3.88%), and stigmast-4-en-3-one (3.50%).

9.8 Quantitative Determination of Total Alkaloid Content in Different Solvent Extracts of *Cymbidium bicolour* Leaves

Sample	Total Alkaloid Content (g/100g)
Acetone Extract	8.02
Chloroform Extract	7.52
Aqueous Extract	8.68
Methanol Extract	11.46
Ethanol Extract	10.12

Table 15: Total Alkaloid content in five Different extracts

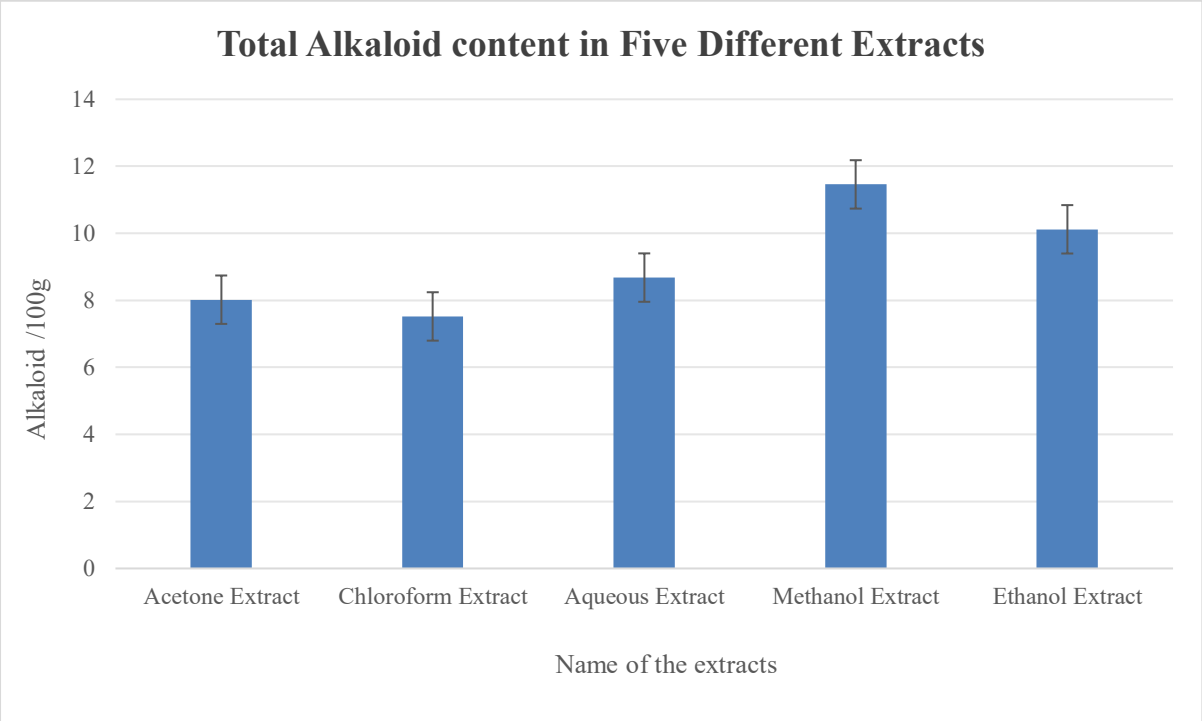


Figure 7: Total alkaloid content (g/100 g) in different solvent extracts of *Cymbidium bicolor* leaves. The methanolic extract exhibited the highest alkaloid content, followed by ethanolic, aqueous, acetone, and chloroform extracts. The results demonstrate the influence of solvent polarity on alkaloid extraction efficiency.

9.9 Quantitative Determination of Total Flavonoid Content in Different Solvent Extracts of *Cymbidium bicolour* Leaves

Quercetin (µg/ml)	Mean OD at 415 nm
Blank	0.052
20	0.259
40	0.476
60	0.744
80	1.006
100	1.307

Table 16: Standard calibration curve for Quercetin

(ISSN: 0973-7049(P))

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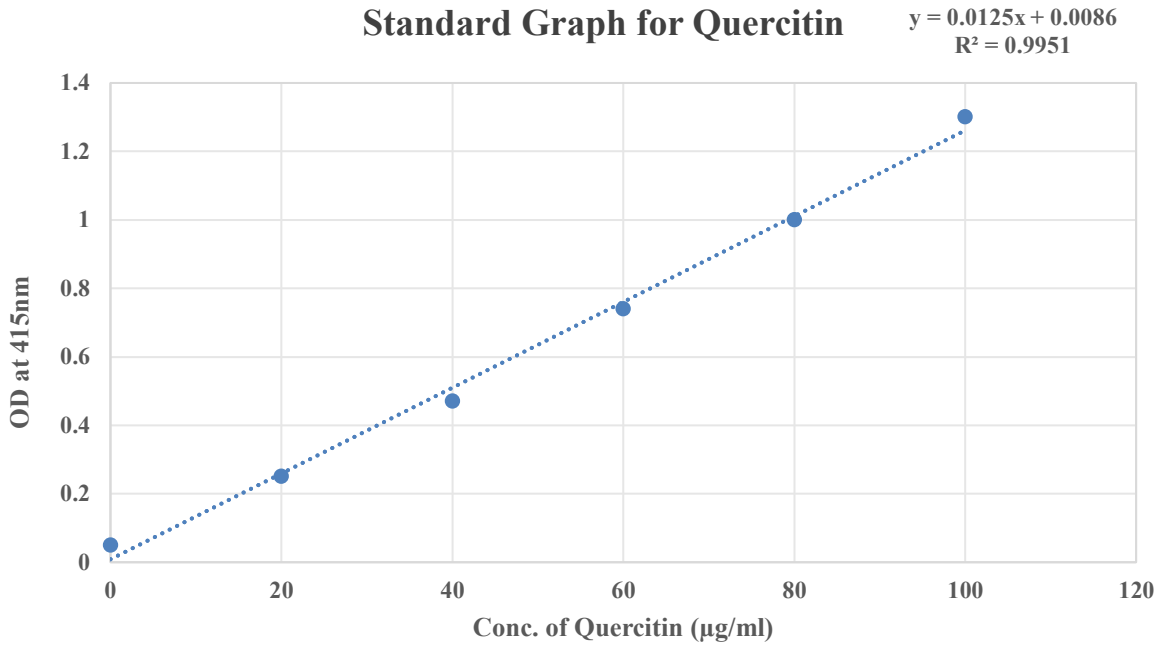


Figure 8: The standard calibration curve for quercetin showed a strong linear relationship between concentration (0–100 µg/ml) and absorbance at 415 nm. The regression equation was y = 0.0125x + 0.0086, with a correlation coefficient (R^2 = 0.9951), indicating excellent linearity and reliability of the method. The increase in absorbance with increasing quercetin concentration confirms the suitability of the standard curve for the quantitative determination of total flavonoid content in the plant extracts.

Sample	Quercetin Equivalent
Aqueous Extract	9.0 µg QE/ml
Chloroform Extract	12.0 µg QE/ml
Acetone Extract	22.0 µg QE/ml
Methanol Extract	29.0 µg QE/ml
Ethanol Extract	33.0 µg QE/ml

Table 17: Total Flavonoid content in five Different extracts expressed as Quercetin Equivalent (µg QE/ml)

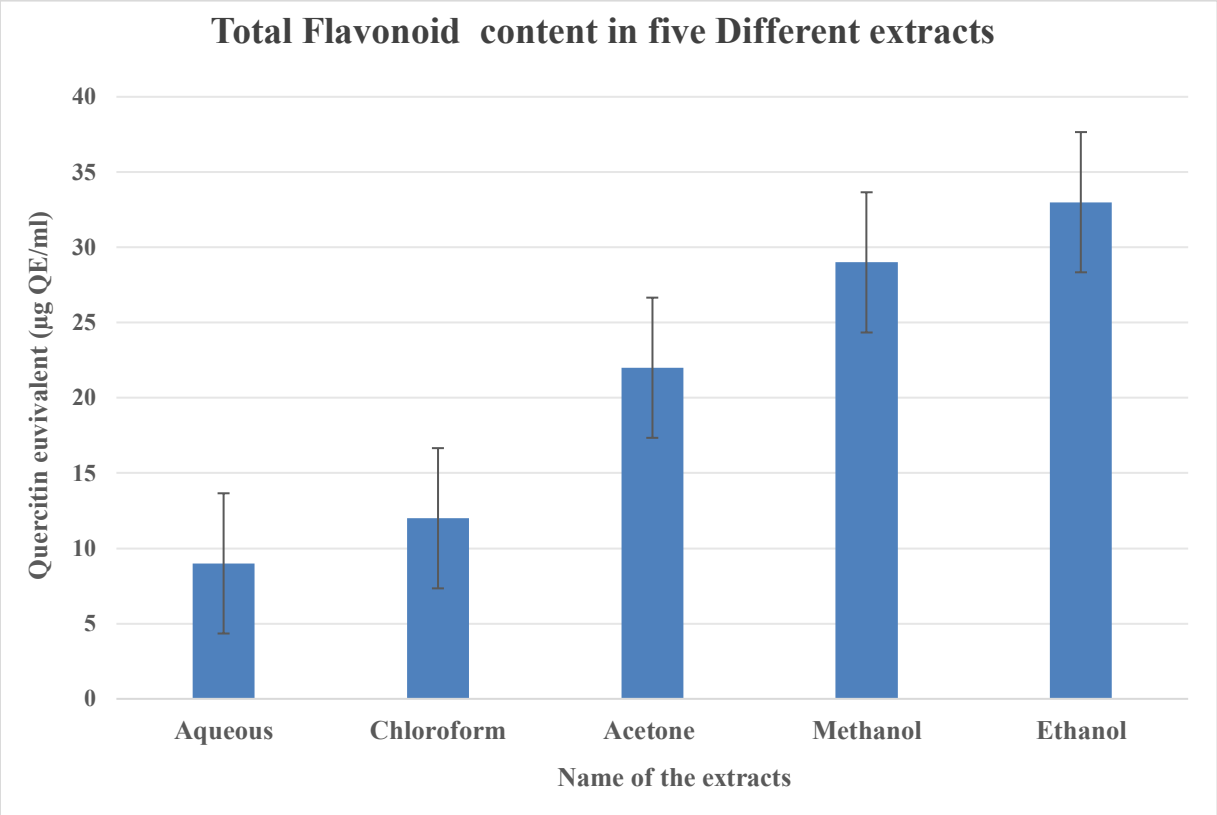


Figure 9: The total flavonoid content of the five different extracts was calculated using the quercetin standard calibration curve and expressed as Quercetin Equivalent (QE). Among the tested extracts, the ethanol extract exhibited the highest flavonoid content (33.0 µg QE/ml), followed by the methanol extract (29.0 µg QE/ml). The water extract showed the lowest flavonoid content (9.0 µg QE/ml

Gallic Acid (µg/mL)	Mean OD
Blank	0.0
20	0.29
40	0.62
60	0.88
80	1.11
100	1.29

Table 18: Standard Curve of Gallic Acid

(ISSN: 0973-7049(P))

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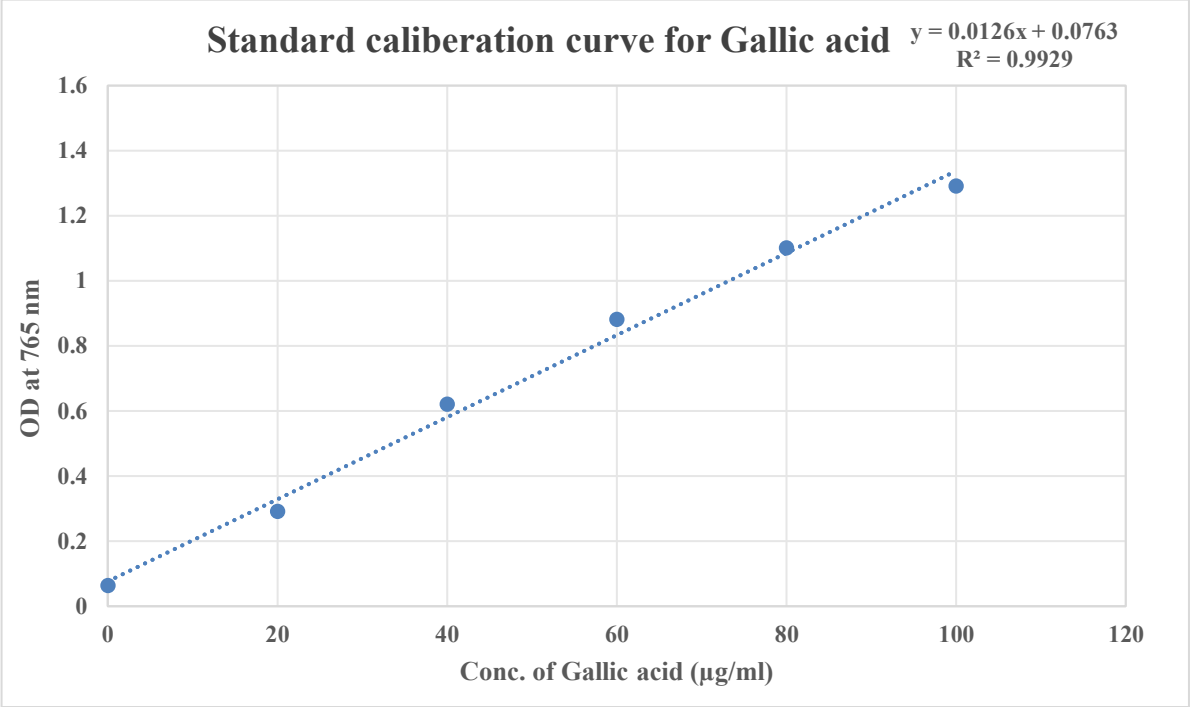


Figure 10: The standard calibration curve of gallic acid was constructed using concentrations ranging from 0–100 µg/ml, and the absorbance was measured at 765 nm. A linear increase in absorbance was observed with increasing concentrations of gallic acid, demonstrating a directly proportional relationship between concentration and optical density. The regression equation obtained was y = 0.0126x + 0.0763, with a coefficient of determination ($R^2 = 0.9929$), indicating a high degree of linearity and precision of the assay. The excellent correlation confirms the reliability of the calibration curve and its suitability for the quantitative estimation of total phenolic content in the plant extracts, expressed as gallic acid equivalents (GAE).

Sample	Phenolic Content
Acetone Extract	125 µg GAE/ml
Chloroform Extract	14 µg GAE/ml
Aqueous Extract	15 µg GAE/ml
Methanol Extract	132 µg GAE/ml
Ethanol Extract	131 µg GAE/ml

Table 19: Total Phenolic content in five Different extracts expressed as Gallic acid equivalents (µg GAE/ml)

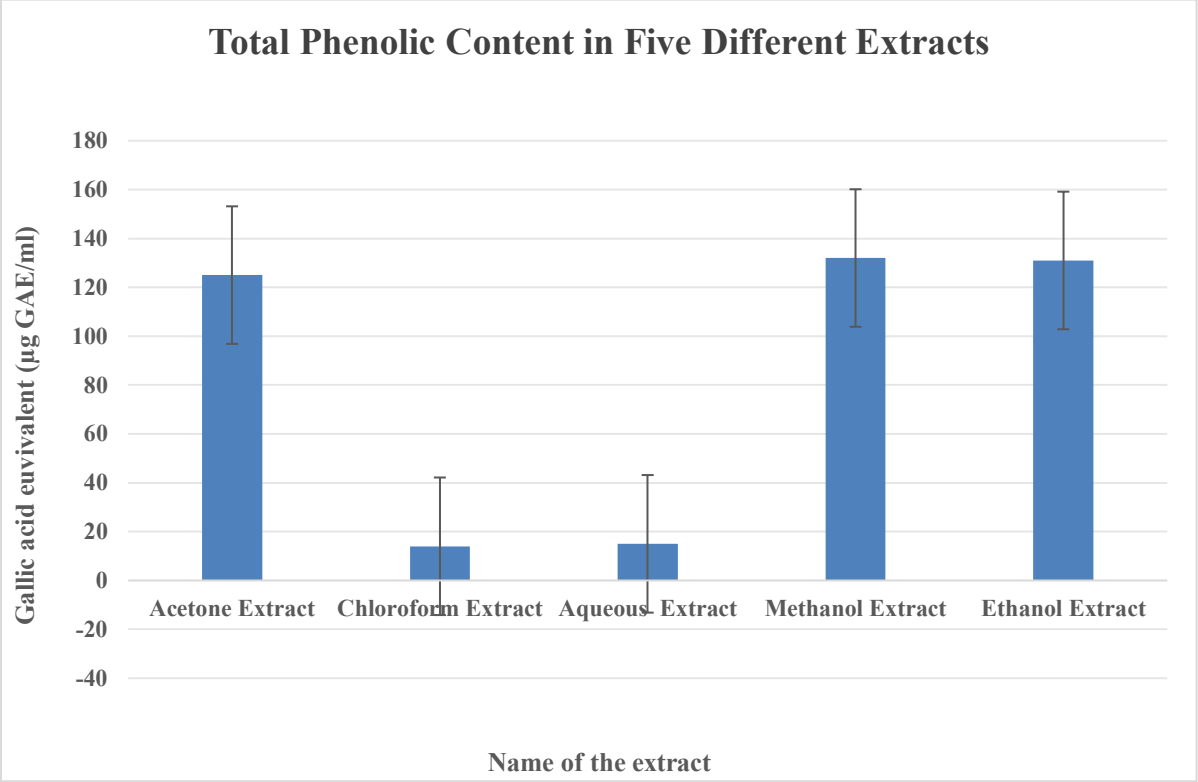


Figure 11: The total phenolic content of the five different extracts was calculated using the gallic acid standard calibration curve and expressed as Gallic Acid Equivalent (GAE). Among the tested extracts, the methanol extract exhibited the highest phenolic content (132 µg GAE/ml), followed by the ethanol extract (131 µg GAE/ml) and acetone extract (125 µg GAE/ml). The aqueous extract (15 µg GAE/ml) and chloroform extract (14 µg GAE/ml) showed comparatively lower phenolic contents, with the chloroform extract exhibiting the lowest value. These results indicate that methanol and ethanol are more efficient solvents for extracting phenolic compounds from *Cymbidium bicolor* leaves.

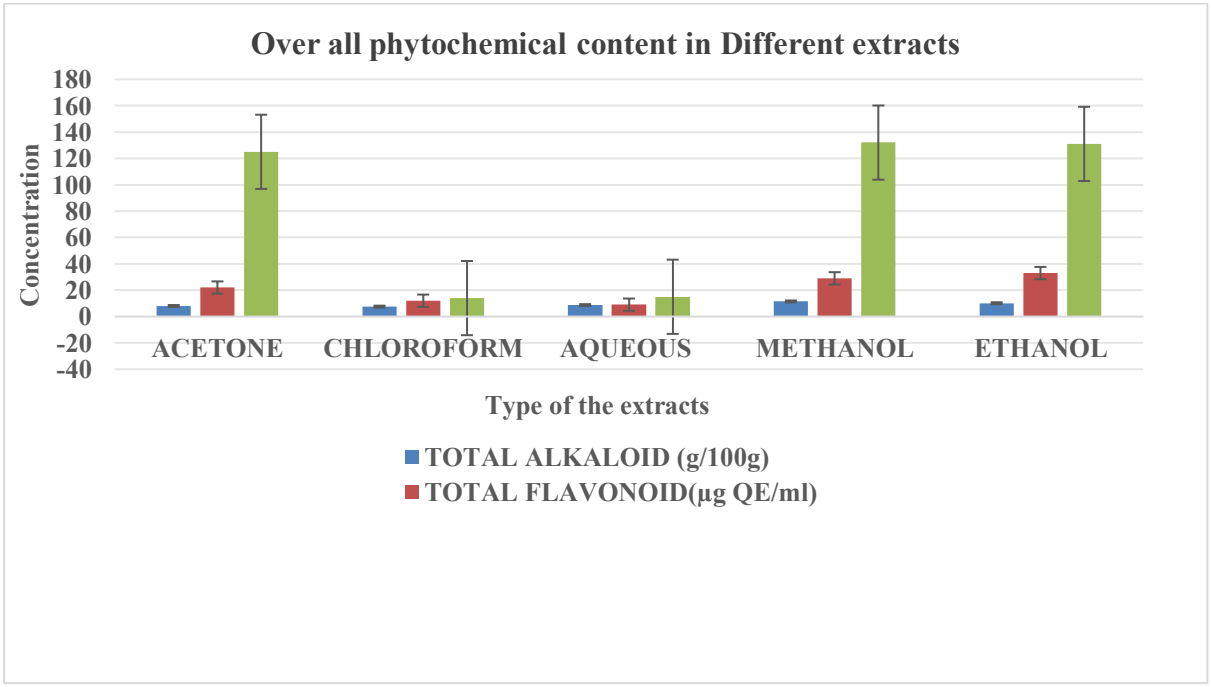


Figure 12: The comparative analysis of phytochemical constituents revealed significant variation among the extracts. The methanol extract contained the highest alkaloid content (11.5 g/100 g), while the ethanol extract showed the highest flavonoid content (33 µg QE/ml). The highest phenolic content was observed in the methanol extract (132 µg GAE/ml), closely followed by the ethanol extract (131 µg GAE/ml). Chloroform and aqueous extracts exhibited comparatively lower phytochemical contents. Overall, methanol and ethanol extracts were found to be the richest sources of bioactive compounds, suggesting their greater potential for antioxidant and therapeutic applications.

9.10 Determination of Antioxidant Activity by DPPH Assay

Concentration (µg/mL)	OD1	OD2	OD3	Mean Absorbance	% Inhibition
0	2.503	2.495	2.509	2.502	0.00%
10	2.282	2.278	2.267	2.276	9.00%
20	1.987	1.976	1.991	1.985	20.70%
40	1.645	1.652	1.681	1.659	33.70%
60	1.287	1.291	1.266	1.281	48.80%
80	0.876	0.852	0.867	0.865	65.40%
100	0.459	0.448	0.43	0.446	82.20%

Table 20: Quercetin was used as a positive control. A standard curve was made and DPPH activity was observed by measuring OD at 517 nm.

Concentration (µg/ml)	Mean Absorbance
0	2.502
10	2.276
20	1.985
40	1.659
60	1.281
80	0.865
100	0.446

Table 21: Standard Curve of Ascorbic Acid (DPPH Assay)

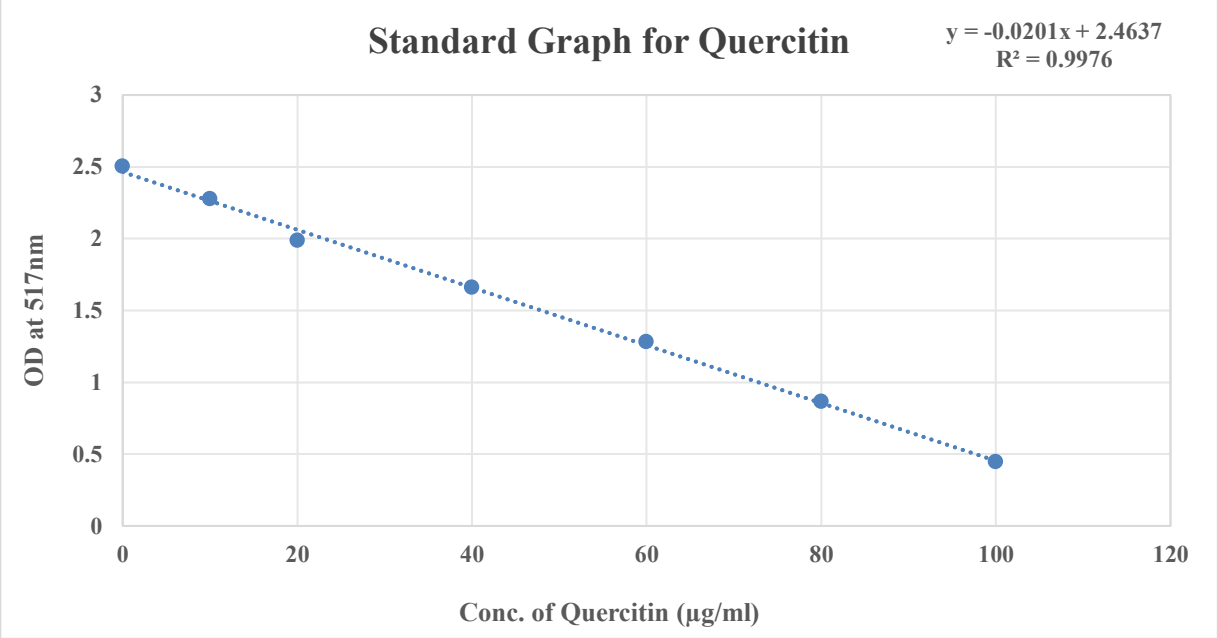


Figure 13: The standard calibration curve of quercetin was constructed by plotting the absorbance values measured at 517 nm against different concentrations of quercetin (0–100 µg/ml). A gradual decrease in absorbance was observed with increasing quercetin concentration, indicating its concentration-dependent antioxidant activity. The calibration curve exhibited excellent linearity with the regression equation y = –0.0201x + 2.4637 and a correlation coefficient (R^2 = 0.9976), demonstrating a strong linear relationship between concentration and absorbance. The high R^2 value confirms the reliability and accuracy of the standard curve for the quantification of flavonoid content in the plant extracts, expressed as quercetin equivalents (QE). The obtained calibration curve was subsequently used to estimate the total flavonoid content of the analysed samples.

Concentration (µg/mL)	% Inhibition
0	0.00%
10	9.00%
20	20.70%
40	33.70%
60	48.80%
80	65.40%
100	82.20%

Table 22: Percentage inhibition of DPPH radical by standard (Quercitin) in various dilutions

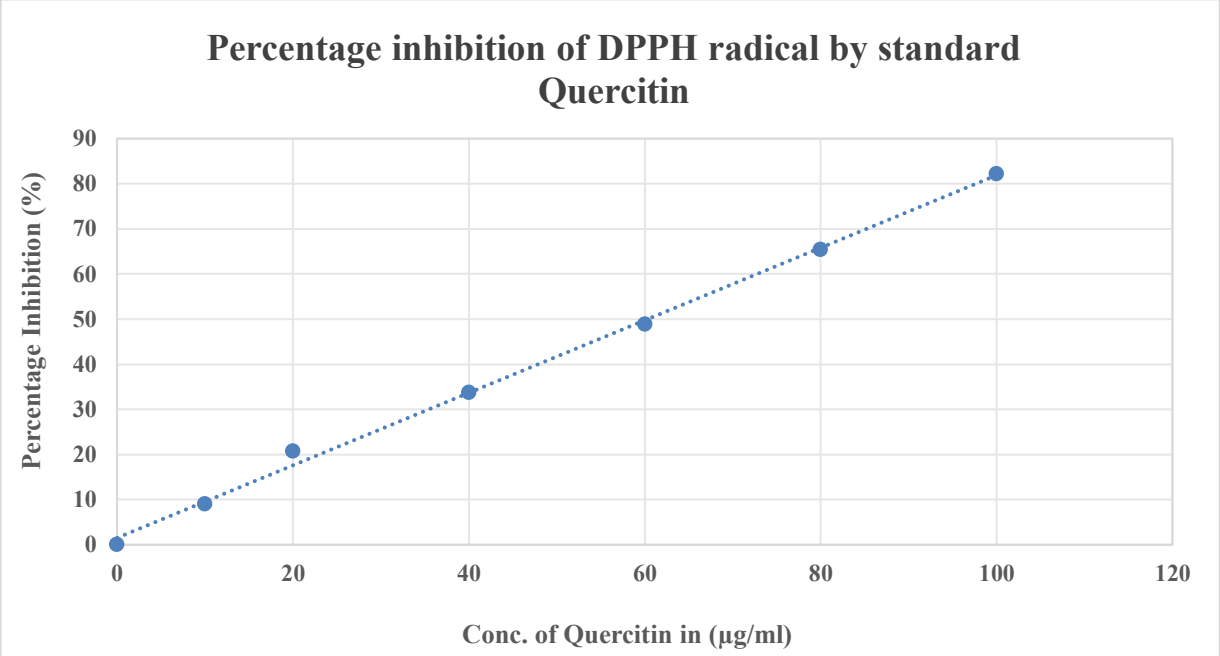


Figure 14: The antioxidant activity of quercetin, used as a reference standard, was evaluated using the DPPH radical-scavenging assay at concentrations ranging from 0 to 100 µg/ml. The percentage of radical inhibition increased progressively with increasing quercetin concentration, indicating a dose-dependent antioxidant effect. The inhibition rate increased from approximately 9% at 10 µg/mL to 82% at 100 µg/mL, demonstrating the strong radical-scavenging potential of quercetin. This concentration-dependent response supports the suitability of quercetin as a reference standard for comparing the DPPH radical-scavenging activity of the tested *Cymbidium bicolor* leaf extracts.

Sample	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	800 µg/ml
Acetone Extract	7.00%	9.50%	10.70%	12.40%	15.30%	19.50%
Water Extract	4.40%	7.50%	8.30%	22.00%	29.10%	48.30%
Chloroform Extract	1.30%	2.20%	4.30%	6.80%	8.70%	15.40%
Ethanol Extract	9.00%	13.90%	11.80%	36.80%	55.70%	60.90%
Methanol Extract	28.30%	29.80%	30.70%	53.80%	64.00%	69.20%

Table 23: Percentage Inhibition of DPPH Radical by Various Extracts

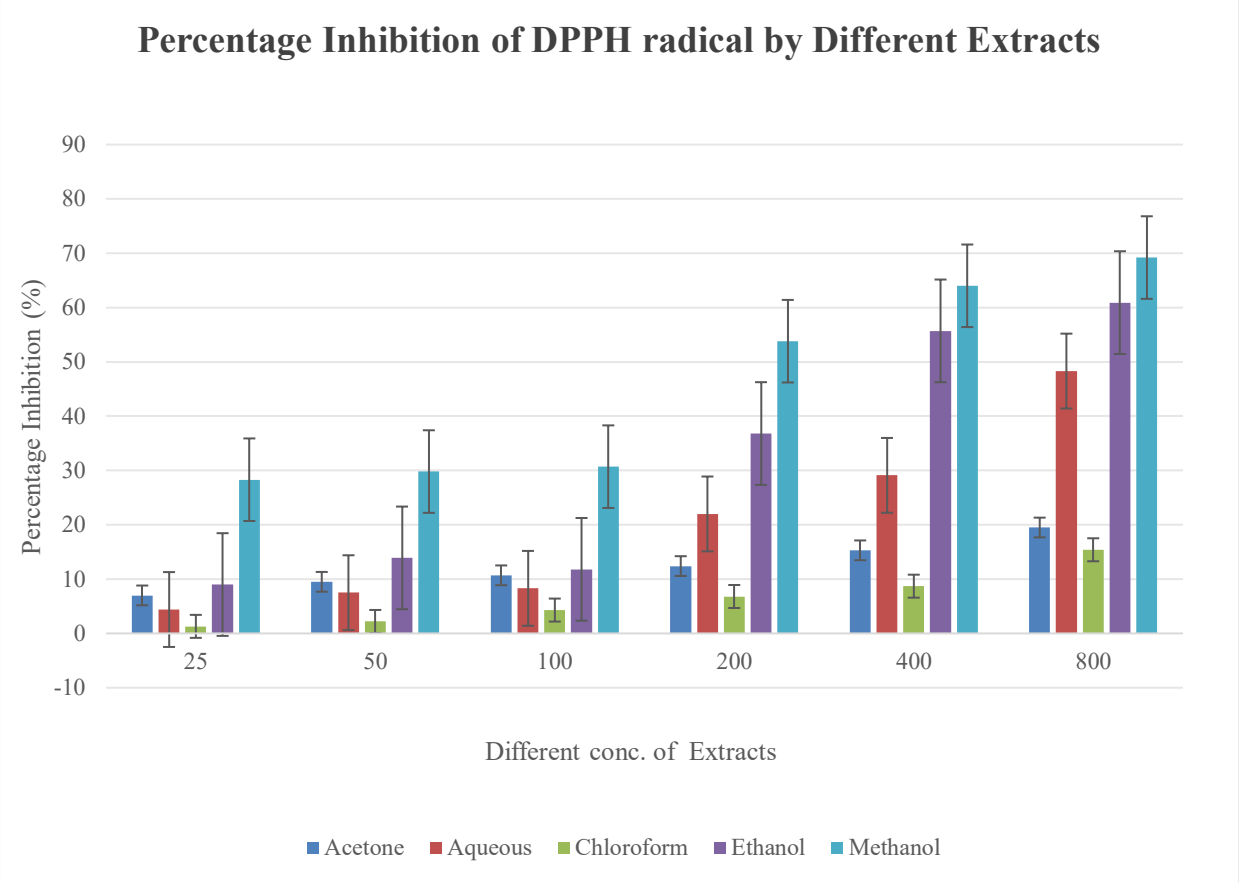


Figure 15: The DPPH radical scavenging activity of the different solvent extracts increased with increasing concentration, indicating a concentration-dependent antioxidant effect. Among the extracts tested, the methanolic extract exhibited the highest antioxidant activity, with percentage inhibition increasing from 28.3% at 25 µg/ml to 69.2% at 800 µg/ml. The ethanolic extract showed the second-highest activity, reaching 60.9% inhibition at 800 µg/ml. The aqueous extract demonstrated moderate antioxidant potential, with inhibition increasing from 4.4% to 48.3% across the tested concentrations. In contrast, the acetone and chloroform extracts exhibited comparatively lower radical scavenging activities, with maximum inhibitions of 19.5% and 15.4%, respectively, at 800 µg/ml. Overall, the antioxidant activity followed the order: Methanol > Ethanol > Water > Acetone > Chloroform, suggesting that methanol was the most effective solvent for extracting antioxidant compounds from the plant material.

9.12 Determination of Antioxidant Activity by ABTS Assay

Trolox Concentration (µg/ml)	OD1	OD2	OD3	Mean Absorbance	% Inhibition
0	0.7	0.704	0.7	0.701	0%
10	0.61	0.63	0.63	0.623	11.10%
20	0.54	0.54	0.52	0.533	24.00%
40	0.46	0.45	0.45	0.453	35.40%
60	0.34	0.36	0.37	0.357	49.10%
80	0.25	0.23	0.26	0.247	64.80%
100	0.11	0.12	0.11	0.113	83.90%

Table 24: Trolox was used as a positive control. A standard curve was made, and ABTS activity was observed by measuring OD at 734 nm.

Trolox Concentration (µg/ml)	Mean Absorbance
0	0.701
10	0.623
20	0.533
40	0.453
60	0.357
80	0.247
100	0.113

Table 25: Absorbance Values of Trolox Standard at Different Concentrations

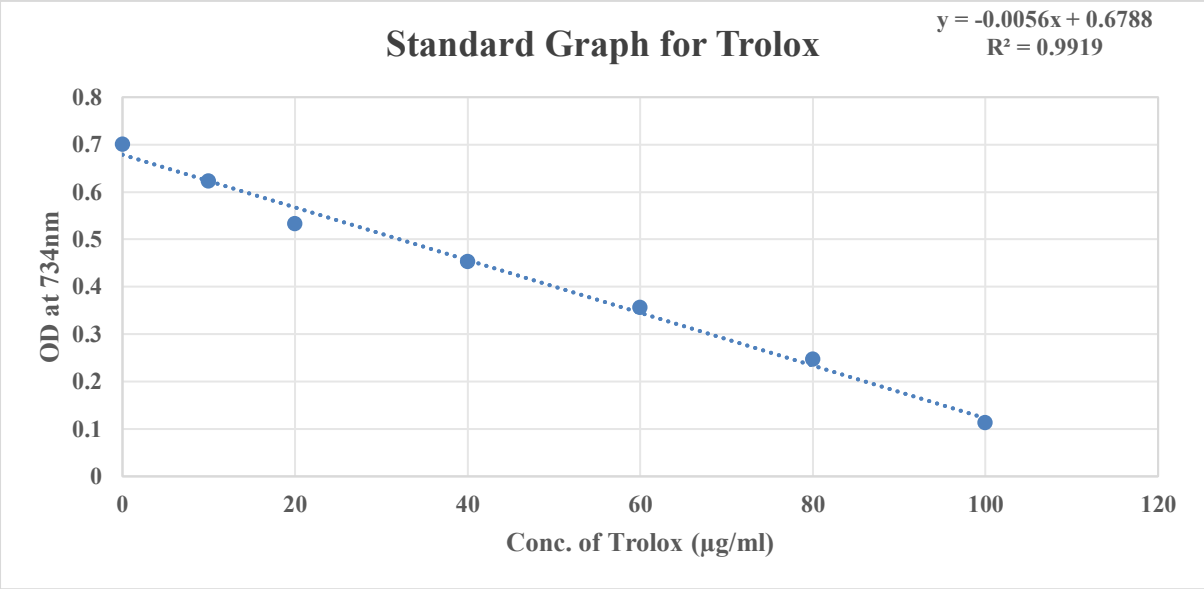


Figure 16: Standard calibration curve of Trolox showing the relationship between concentration and absorbance at 734 nm. The absorbance at 734 nm decreased with increasing Trolox concentration, indicating a concentration-dependent antioxidant effect. A strong linear relationship was observed with the regression equation $y = -0.0056x + 0.6788$ and $R^2 = 0.9919$, confirming the accuracy of the standard curve. This calibration curve was used to determine the antioxidant activity of the extracts and express the results as Trolox equivalents.

Trolox Concentration (µg/ml)	% Inhibition
0	0%
10	11.10%
20	24.00%
40	35.40%
60	49.10%
80	64.80%
100	83.90%

Table 26: Percentage inhibition of ABTS radical by standard (Trolox) in various dilutions

(ISSN: 0973-7049(P))

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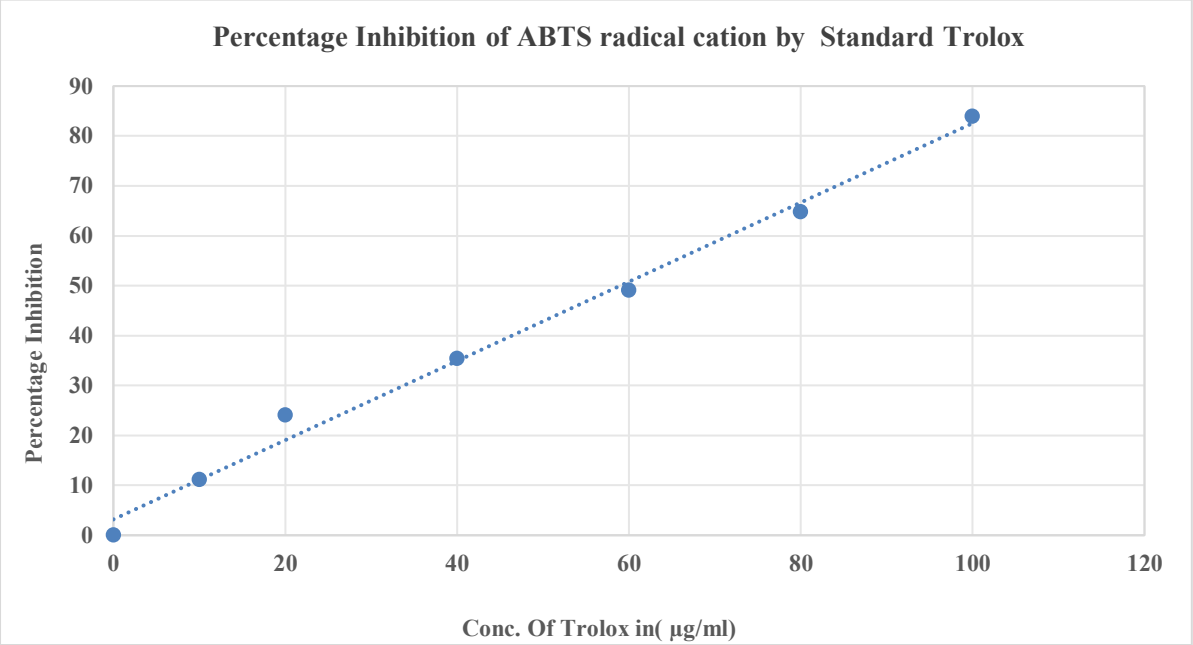


Figure 17: The ABTS radical-scavenging activity of Trolox, used as a reference standard, was evaluated at concentrations ranging from 0 to 100 µg/ml. The percentage of radical inhibition increased progressively with increasing Trolox concentration, reaching approximately 84% at 100 µg/ml. This concentration-dependent response demonstrates the antioxidant capacity of Trolox and establishes its suitability as a reference standard for evaluating the ABTS radical-scavenging activity of the tested *Cymbidium bicolor* leaf extracts.

Sample	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	800 µg/ml
Acetone Extract	1.10%	3.30%	7.50%	9.70%	12.80%	20.60%
Chloroform Extract	2.60%	6.00%	7.70%	9.50%	12.50%	15.10%
Methanol Extract	2.50%	25.60%	28.80%	31.00%	32.40%	61.60%
Ethanol Extract	2.00%	9.50%	24.00%	31.10%	54.70%	69.60%
Aqueous Extract	1.40%	3.80%	7.20%	10.20%	23.00%	50.00%

Table 27: Percentage Inhibition of ABTS radical by Five Different extracts at Different concentrations

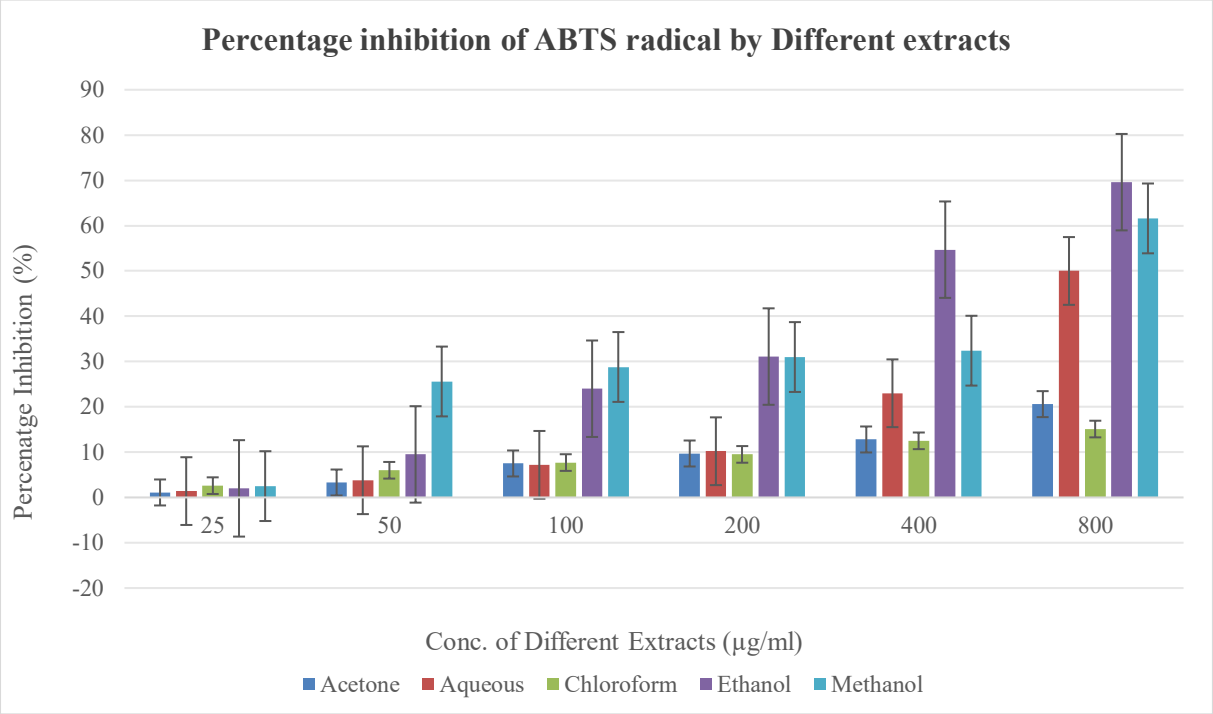


Figure 18: The ABTS radical scavenging activity of the different solvent extracts of *Cymbidium bicolor* increases with increasing concentration (25–800 µg/ml). Among the extracts tested, the ethanol extract shows the highest antioxidant activity, showing 69.7% inhibition at 800 µg/ml, followed by the methanol extract with 61.6% inhibition. The aqueous extract demonstrated moderate activity, reaching 50.0% inhibition at the highest concentration. In contrast, the acetone and chloroform extracts showed comparatively fewer scavenging activities, with the maximum inhibition values of 20.5% and 15.1%, respectively. The results show that ethanol and methanol is more effective solvents for getting antioxidant, compounds from the *Cymbidium bicolor* leaves.

Discussion

The present study investigated the phytochemical composition and antioxidant potential of *Cymbidium bicolor* leaf extracts prepared using five different solvents: methanol, ethanol, water, acetone, and chloroform. The extraction yield varied considerably among the solvents, with methanol exhibiting the highest yield (13.76%), followed by chloroform (11.56%), aqueous (10.00%), ethanol (5.28%), and acetone (5.08%). These variations highlight the influence of solvent polarity and extraction efficiency on the recovery of plant constituents. Qualitative phytochemical screening indicated the presence of various secondary metabolites, including flavonoids and terpenoids. Quantitative analysis revealed that the methanolic extract exhibited the highest total alkaloid content (11.46 g/100 g) and total phenolic content (132 µg/ml), whereas the ethanolic extract showed the highest total flavonoid content (33 µg/mL). In the DPPH radical-scavenging assay, the methanolic extract demonstrated the highest activity (69.2%), followed by the ethanolic (60.9%), aqueous (48.3%), acetone (19.5%), and chloroform (15.4%) extracts at 800 µg/ml. Similarly, the ABTS assay showed the highest radical-scavenging activity in the ethanolic extract (69.6%). The differences observed between the DPPH and ABTS assays may be attributed to variations in phytochemical composition and the distinct reaction mechanisms of the assays. Furthermore, GC–MS analysis revealed a diverse range of chemical constituents, including phytol, stigmasterol, β-sitosterol, tocopherols, and other compounds of potential biological

interest. Among the selected GC–MS peaks, bis(2-ethylhexyl) phthalate (15.30%), β-sitosterol (13.86%), and δ-tocopherol (12.31%) exhibited the highest relative peak areas; however, potential contaminants and library-based identifications require further verification. Collectively, these findings suggest that methanol and ethanol are promising extraction solvents for recovering constituents associated with the antioxidant activity of *C. bicolor*. Further investigations are required to establish the contribution of individual compounds to the observed antioxidant effects.

Conclusion

The study provides preliminary evidence of the phytochemical diversity and antioxidant potential of *Cymbidium bicolor* leaves through solvent extraction, qualitative and quantitative phytochemical analysis, DPPH and ABTS radical-scavenging assays, and GC–MS profiling. Among the five solvents investigated, methanol produced the highest extraction yield (13.76%), total alkaloid content (11.46 g/100 g), and total phenolic content (132 µg/mL). In contrast, ethanol exhibited the highest total flavonoid content (33 µg/mL) and ABTS radical-scavenging activity (69.6%). The methanolic extract demonstrated the highest DPPH radical-scavenging activity (69.2%) at 800 µg/ml, indicating its promising antioxidant potential under the experimental conditions. GC–MS analysis further revealed several putative chemical constituents, including phytol, stigmasterol, β-sitosterol, and tocopherols, which warrant further investigation. Overall, these results establish a preliminary basis for future research into the biological properties of *C. bicolor* leaf extracts. Further studies involving cytotoxicity evaluation against cancer and normal cell lines, IC_{50} determination, apoptosis assays, and isolation and structural characterisation of individual compounds are necessary to assess its potential anticancer properties and elucidate the mechanisms underlying its biological activity.

References

1. Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. (2022). Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa. *Molecules*, *27*(4), 1326. https://doi.org/10.3390/molecules27041326
2. Chugh, S., Guha, S., & Rao, I. U. (2009). Micropropagation of orchids: A review on the potential of different explants. *Scientia Horticulturae*, *122*(4), 507–520. https://doi.org/10.1016/j.scienta.2009.07.016
3. Dauda, H., Uba, G., & Ali, U. (2020). Preliminary Phytochemical Screening, Quantitative Analysis of Flavonoids from the Stem Bark Extract of Commiphora africana (Burseraceae). *Bulletin of Environmental Science and Sustainable Management*, *4*(1), 25–27. https://doi.org/10.54987/bessm.v4i1.529
4. Elbouzidi, A., Haddou, M., Baraich, A., Taibi, M., Hachlafi, N. E., Pareek, A., Mesnard, F., & Addi, M. (2025). Biochemical insights into specialised plant metabolites: Advancing cosmeceutical applications for skin benefits. *Journal of Agriculture and Food Research*, *19*, 101651. https://doi.org/10.1016/j.jafr.2025.101651

5. Gil-Martín, E., Forbes-Hernández, T., Romero, A., Cianciosi, D., Giampieri, F., & Battino, M. (2021). Influence of the extraction method on the recovery of bioactive phenolic compounds from food industry by-products. *Food Chemistry*, *378*, 131918. https://doi.org/10.1016/j.foodchem.2021.131918
6. Joshi, R., Satyal, P., & Setzer, W. (2016). Himalayan Aromatic Medicinal Plants: A Review of their Ethnopharmacology, Volatile Phytochemistry, and Biological Activities. *Medicines*, *3*(1), 6. https://doi.org/10.3390/medicines3010006
7. Kaundal, R., & Kumar, D. (2025). Current demands for standardisation of Indian medicinal plants: A critical review. *Medicine in Drug Discovery*, *27*, 100211. https://doi.org/10.1016/j.medidd.2025.100211
8. Krakowska-Sieprawska, A., Kiełbasa, A., Rafińska, K., Ligor, M., & Buszewski, B. (2022). Modern methods of Pre-Treatment of plant material for the extraction of bioactive compounds. *Molecules*, *27*(3), 730. https://doi.org/10.3390/molecules27030730
9. Kumar, A., P N. Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M., Elobeid, T., K S., & Oz, F. (2023). Major Phytochemicals: recent advances in health benefits and extraction methods. *Molecules*, *28*(2), 887. https://doi.org/10.3390/molecules28020887
10. Letchuman, S., Madhuranga, H. D., Kaushalya, M., Premarathna, A. D., & Saravanan, M. (2024). Alkaloids unveiled: A comprehensive analysis of novel therapeutic properties, mechanisms, and plant-based innovations. *Intelligent Pharmacy*, *3*(4), 268–276. https://doi.org/10.1016/j.ipha.2024.09.007
11. Lucas, B. N., Nora, D., Boeira, C. P., Verruck, S., & Da Rosa, C. S. (2022). Determination of total phenolic compounds in plant extracts via Folin-Ciocalteu's method adapted to the usage of digital images. *Food Science and Technology*, *42*. https://doi.org/10.1590/fst.35122
12. Nicolescu, A., Bunea, C. I., & Mocan, A. (2025). Total flavonoid content revised: An overview of past, present, and future determinations in phytochemical analysis. *Analytical Biochemistry*, *700*, 115794. https://doi.org/10.1016/j.ab.2025.115794
13. Ozgen, M., Reese, R. N., Tulio, A. Z., Scheerens, J. C., & Miller, A. R. (2006). Modified 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) methods. *Journal of Agricultural and Food Chemistry*, *54*(4), 1151–1157. https://doi.org/10.1021/jf051960d

14. Pandey, M. M., Rastogi, S., & Rawat, A. K. S. (2013). Indian Traditional Ayurvedic system of medicine and nutritional supplementation. *Evidence-based Complementary and Alternative Medicine*, *2013*, 1–12. https://doi.org/10.1155/2013/376327
15. Ram, N. H. (2023). Increasing Extinction of Plant Species of Plants in India - Alarming Situation. *RESEARCH HUB International Multidisciplinary Research Journal*, *10*(2), 95–102. https://doi.org/10.53573/rhimrj.2023.v10n02.020
16. Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603–608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834
17. Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603–608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834
18. Simón-Manso, Y., Lowenthal, M. S., Kilpatrick, L. E., Sampson, M. L., Telu, K. H., Rudnick, P. A., Mallard, W. G., Bearden, D. W., Schock, T. B., Tchekhovskoi, D. V., Blonder, N., Yan, X., Liang, Y., Zheng, Y., Wallace, W. E., Neta, P., Phinney, K. W., Remaley, A. T., & Stein, S. E. (2013). Metabolite profiling of a NIST Standard reference material for human plasma (SRM 1950): GC-MS, LC-MS, NMR, and clinical laboratory analyses, libraries, and Web-Based resources. *Analytical Chemistry*, *85*(24), 11725–11731. https://doi.org/10.1021/ac402503m
19. Tiwari, P., Bose, S. K., Gautam, A., & Chen, J. (2023b). Emerging trends and insights into the cultivation strategies, ethnomedicinal uses, and socio-economic attributes of orchids. *The Journal of Horticultural Science and Biotechnology*, *98*(3), 273–298. https://doi.org/10.1080/14620316.2022.2164524
20. Tiwari, P., Sharma, A., Bose, S. K., & Park, K. (2024). Advances in Orchid biology: biotechnological achievements, translational success, and commercial outcomes. *Horticulturae*, *10*(2), 152. https://doi.org/10.3390/horticulturae10020152
21. Van Tan, P. (2018b). The Determination of Total Alkaloid, Polyphenol, Flavonoid and Saponin Contents of Pogang gan (Curcuma sp.). *International Journal of Biology*, *10*(4), 42. https://doi.org/10.5539/ijb.v10n4p42