

Phytochemical Profiling, Antifungal Potential of *Vinca rosea* Extracts and Isolated Compound Against Wheat Pathogens from Northern Part of India

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Abstract

Natural products significantly impact the pharmaceutical and agricultural industries, especially as alternatives to synthetic fungicides. Our research aimed to investigate the phytochemical properties of the *V. rosea* plant, focusing on extracts obtained from various polar and non-polar solvents and their antifungal potential against two fungi affecting wheat plants (*Fusarium graminearum* and *Bipolaris sorokiniana*). We used multiple techniques (maceration, sonication, Soxhlet extraction) to prepare extracts, and their phytochemical screening was done to analyze different bioactive compounds, which was further confirmed by gas chromatography-mass spectrometry (GC-MS), showing the presence of alkaloids, phenols, flavonoids, tannins, and saponins as bioactive compounds in this plant. GC-MS analysis helped identify potentially important compounds with higher concentrations and further helped to isolate vindoline from leaf extract. Antifungal assays demonstrated vindoline effectiveness against *F. graminearum* and *B. sorokiniana*. This study's findings underscore the importance of *V. rosea* as a natural source of antifungal agents. The identified bioactive compounds in GC-MS analysis also pave the way for developing pharmaceutical biosynthesis pathways, enlightening future research and development in these industries.

INTRODUCTION

In recent years, the widespread use of synthetic fungicides for managing agricultural pathogens has raised substantial environmental and health concerns, highlighting the need for safer, more sustainable alternatives. Natural substances derived from plants, such as extracts and essential oils, have gained attention due to their diverse bioactivities, which play essential roles in medicine and agriculture.[1] Various plant parts contain a rich array of phytochemicals with broad biological activities, and both agricultural and pharmaceutical industries have utilized these compounds to develop numerous drugs and agrochemicals.[2] Among these, medicinal plants have emerged as promising candidates, offering bioactive compounds with significant therapeutic and agricultural potential.[1, 3]

One such plant is *V. rosea* (*Catharanthus roseus*), a member of the Apocynaceae family, and native to Madagascar and widely cultivated for its medicinal properties. In India, it is known as Sadabahar and Nityakalyani and is commonly grown in regions such as Punjab, Kerala, Gujarat, Uttar Pradesh, and Assam. Traditionally, *V. rosea* has been used to treat conditions like high blood pressure, diabetes, and wasp stings.[4, 5, 6] The plant's terpenoid indole alkaloid pathway synthesizes over 130 alkaloids, including commercially important vinca alkaloids like vincristine and vinblastine, used as anticancer drugs, and ajmalicine, an antihypertensive agent approved by the World Health Organization.[7–12]

Despite its traditional medicinal use and the commercial cultivation of its alkaloids for cancer treatment, comprehensive research on the phytochemical composition and antifungal potential of *V. rosea* extracts, especially against wheat pathogens (*Fusarium graminearum* and *Bipolaris sorokiniana*), remains limited. Most studies have focused on methanolic extracts from the plant's leaves and flowers.[9–15] However, a comparative analysis of different solvent extracts and their specific bioactive compounds is lacking. *V. rosea* extracts have been reported to contain various compounds, including phenols, flavonoids, anthocyanins, volatile compounds, and alkaloids. The plant is recognized for its diverse applications in agriculture and pharmacology, exhibiting anthelmintic, antioxidant, antimicrobial, antibacterial, antifungal, and antidiarrheal activities. Key constituents such as vindoline, vincristine, vinblastine, catharanthine, leurosine, syringic acid, petunidin, quercetin, para-coumaric acid, heneicosane, tricosane, and palmitic acid are responsible for its bioactivity in both agronomic and therapeutic contexts.[16, 17]

The phytochemical profile of *V. rosea* can vary depending on geographical and climatic conditions.[18, 19] This study addresses the knowledge gap by conducting a detailed phytochemical analysis of the plant from Northern India.

Limited research has been reported on the antifungal activity of *V. rosea* extracts against phytopathogenic fungi.[7, 9, 20] *Fusarium graminearum*, a major fungal pathogen, causes billions of dollars in losses worldwide by altering the amino acid composition in wheat, producing shriveled kernels, and contaminating grains with mycotoxins such as deoxynivalenol, which disrupts protein synthesis.[21, 22] Another significant pathogen, *Bipolaris sorokiniana*, is responsible for diseases in cereals, leading to millions of tons of wheat loss annually.[23, 24, 25]

To explore the relationship between the active ingredients and their biological activity, this study evaluates the antifungal activity of *V. rosea* leaf and flower extracts and isolated compound against pathogenic wheat fungi—specifically *F. graminearum*, which causes Fusarium head blight, and *B. sorokiniana*, which leads to spot blotch and root rot in wheat. The findings aim to contribute to developing natural, plant-based fungicides as sustainable alternatives to synthetic chemicals in agriculture.

EXPERIMENTS AND METHODS

Plant Collection and Authentication

Vinca rosea leaves were harvested in the spring from the botanical gardens of Punjab Agricultural University (PAU) in Ludhiana, Punjab. The precise location of the collection is at coordinates 30.900965°N latitude 75.857277°E longitude. The plant species was formally identified and authenticated by the Principal Scientist and Head of the Department of Floriculture and Landscaping at PAU, with an authentication certificate provided for reference (refer to SI). The authors confirm that all methods were carried out by relevant guidelines and regulations of Punjab Agricultural University, Ludhiana. The collection of the plants used in the study complies with local or national guidelines with no need for further affirmation.

Pathogen Cultures

Cultures of two phytopathogenic fungi, *Fusarium graminearum* and *Bipolaris sorokiniana*, were obtained from Dr. Jaspal Kaur, a Senior Plant Pathologist at PAU. These fungal strains were maintained on slants of potato dextrose agar (PDA) and stored at 4°C to preserve viability.

Experiments

The supporting information details all the experiments conducted, including quantitative analysis, extract preparation, phytochemical testing, GC-MS analysis, compound isolation, and antifungal activity assessment. It also thoroughly covers the methodologies used to obtain the data reported in the research and the instrumental details employed for the GC-MS analysis. (Refer to SI for detailed procedure)

RESULT AND DISCUSSION

Quantitative analysis

The quantitative analysis of *V. rosea* leaves and flower powder was conducted to determine the content of alkaloids, phenols, flavonoids, tannins, saponins, and glycosides, with results in Table 1. The categories used for quantitative analysis were selected based on the literature, which enlists its main categories of compounds.[27] The alkaloid content was the highest among the phytoconstituents, with the leaves containing 13.25% and the flowers 5.55%. This high concentration of alkaloids in *V. rosea*, particularly vindoline, explains its widespread medicinal use in cancer

treatment.[28, 29, 30, 31] The alkaloid content in the leaves aligns closely with previous findings by Esther et al. [27], which reported $11.84 \pm 1.2\%$.

Table 1 Quantitative analysis of <i>V. rosea</i> leaves and flower powder		
Composition	Leaves	Flowers
Alkaloid	13.25 ± 0.4242	5.55 ± 0.4738
Phenol	0.32 ± 0.0141	0.42 ± 0.0358
Tannin	0.75 ± 0.0282	0.85 ± 0.0498
Flavonoid	0.51 ± 0.0424	0.63 ± 0.0568
Saponin	0.47 ± 0.0282	0.35 ± 0.0498
Glycoside	1.815 ± 0.0919	0.72 ± 0.0218
The results were expressed as Mean $\pm$ Standard deviation.		

Phenolic content was measured at 0.32 mg TAE/g dw in leaves and 0.42 mg TAE/g dw in flowers, while tannin content was 0.75 mg TAE/g dw in leaves and 0.85 mg TAE/g dw in flowers. These results are consistent with Esther et al [27] and Agarwal et al,[11] which reported a phenol content of 0.32 ± 0.01 mg TAE/g dw and a tannin content of 0.68 ± 0.3 mg TAE/g dw in leaves. Flavonoid content was higher in flowers (0.63 mg QE/g dw) compared to leaves (0.51 mg QE/g dw), and these findings are similar to those of Esther et al.[27], which reported 0.48 ± 1.3 mg QE/g dw in leaves. Saponin content was found to be 0.47% in leaves and 0.35% in flowers, with leaf saponin content closely matching the $0.46 \pm 0.6\%$ reported by Esther et al.[27] Glycoside content was 1.815% in leaves and 0.72% in flowers.

These findings highlight the significant presence of bioactive compounds in *V. rosea*, with leaves showing higher concentrations of most phytoconstituents than flowers. This detailed compositional analysis underscores the plant's potential for diverse medicinal and therapeutic applications, justifying its extensive use in traditional and modern medicine. The standard curves of tannic acid and quercetin, provided in Figs. 1 and 2, respectively, validate the accuracy and reliability of the quantification methods used in this study.

Comparison of yield of extracts

Plant extracts are crucial in isolating bioactive compounds from plant materials, essential for developing various drugs.[29] The extracts of *V. rosea* leaves and flowers were prepared using three different extraction methods—maceration, Soxhlet, and sonication—and four solvents: methanol, acetone, chloroform, and hexane (Table 2). Among these solvents, the methanolic extract of leaves showed the highest yield (9.3%) using the maceration technique. In comparison, using the Soxhlet extraction method, the methanolic extract of flowers had the highest yield (9.6%). This indicates that polar solvents like methanol generally produce higher yields than non-polar solvents like petroleum ether and hexane, irrespective of the extraction method. These findings are consistent with those reported by Adhikari et al, [23] who observed higher yields of *Taxus wallichiana* extracts in polar solvents compared to non-polar ones, and similar results were also reported by Kapoor and Rani [16] and Goswami.[30]

Table 2
Yield of extracts obtained from leaves and flowers of *V. rosea* plant

Extract	MA	SX	SO ^a	SO ^b	SO ^c	MA	SX	SO ^a	SO ^b	SO ^c
Leaves					Flowers					
Methanol	9.3	8.5	8.2	8.4	8.7	8.9	9.6	8.5	8.8	9.1
Acetone	8	7.6	6.9	7.3	7.7	7.8	8.3	7.2	7.5	7.8
Chloroform	5	3.8	2.8	3.4	3.9	4.1	5.3	3.9	4.4	4.8
Hexane	4.4	4.5	2.1	2.8	3.6	3.9	5.7	3.3	3.7	4.1
The data mentioned above are expressed in Percentage (%).										
MA- Maceration (48 h), SX- Soxhlet (24 h), SO-Sonication (10 ^a , 20 ^b and 30 ^c min)										

The extracts' yield increased with the solvents' polarity, following the order: methanol > acetone > chloroform > hexane. The yield varied significantly depending on the solvent and the extraction technique used. Although the fastest method, sonication generally produced lower yields than maceration and Soxhlet methods. Maceration and Soxhlet, which require 48 hours and 24 hours, respectively, produced higher yields despite the longer extraction times. These results suggest that while sonication offers a quicker extraction process, the choice of solvent and extraction technique significantly influences the yield of plant extracts, with polar solvents and longer extraction times generally providing better results, which could be due to extracting more compounds via cold press methods.

Phytochemical analysis

Phytochemical studies are considered a practical approach for discovering the bioactive profiles of therapeutically important plants.[31] In the present study, extracts from the leaves and flowers of *V. rosea* were subjected to phytochemical screening to identify various chemical constituents, including alkaloids, amino acids, carbohydrates, flavonoids, glycosides, phenols, saponins, tannins, terpenoids, and cardiac glycosides. The results of the qualitative analysis of these extracts are presented in Table 3. The bioactive constituents, such as alkaloids, amino acids, carbohydrates, and glycosides, were found in polar solvents like methanol and acetone. At the same time, they were absent in non-polar solvents like chloroform and hexane. Phenols and flavonoids were consistently present in all extracts. Notably, the methanolic extract of leaves and flowers showed the highest detection of bioactive compounds, whereas the hexane extract revealed the fewest. These findings align with previous studies by Aziz et al. [26] and Kapoor and Rani.[16] Acetone extracts of both leaves and flowers showed an absence of carbohydrates, glycosides, and cardiac glycosides. The data from this study are valuable for further separating and identifying compounds from various extracts of *V. rosea*, providing a foundation for further research into its potential.

Table 3
Phytochemical analysis of different extracts obtained from *V. rosea* leaves and flowers

Constituents	Methanol	Acetone	Chloroform	Hexane	Methanol	Acetone	Chloroform	Hexane
	Leaves				Flowers			
Alkaloids	+	+	-	-	+	+	-	-
Amino Acid	+	+	-	-	+	+	-	-
Carbohydrates	+	-	-	-	+	-	-	-
Flavonoids	+	+	+	+	+	+	+	+
Glycosides	+	-	-	-	+	-	-	-
Phenol	+	+	+	+	+	+	+	+
Saponin	+	+	-	+	+	+	-	-
Tannin	+	+	-	+	+	+	+	+
Terpenoid	+	+	+	+	+	+	-	+
Cardiac Glycosides	+	-	-	-	+	-	-	-
(+) = Presence and (-) = Absence								

Total phenolic content and total flavonoid content

The total phenolic content (TPC) and total flavonoid content (TFC) of different extracts from the leaves and flowers of *V. rosea* were determined using standard calibration curves of gallic acid ($y = 0.43818x + 0.00964$, $r^2 = 0.997$) and quercetin ($y = 0.5203x + 0.00673$, $r^2 = 0.9995$), respectively. The results are summarized in Table 4. The methanolic extracts of both leaves and flowers exhibited the highest phenolic content compared to other solvents. Specifically, the TPC in the methanolic leaf extract was 29.92 ± 0.0848 mg GAE/g dry weight, while the acetone, chloroform, and hexane extracts had TPC values of 28.055 ± 0.0636 , 18.15 ± 0.2121 , and 16.06 ± 0.0707 mg GAE/g dry weight, respectively. Similar trends were observed in the flower extracts, with the methanolic extract having a TPC of 33.62 ± 0.1414 mg GAE/g dry weight, followed by acetone (30.495 ± 0.4030), chloroform (20.395 ± 0.0919), and hexane (18.53 ± 0.3959) extracts. These findings align with previous research by Kapoor and Rani.[16]

Table 4
TPC and TFC of different extracts of leaves and flowers of *Vinca rosea*

	Methanol	Acetone	Chloroform	Hexane	Methanol	Acetone	Chloroform	Hexane
	Leaves				Flowers			
TPC	29.92 ± 0.084	28.05 ± 0.063	18.15 ± 0.212	16.06 ± 0.070	33.62 ± 0.141	30.49 ± 0.403	20.39 ± 0.091	18.53 ± 0.395
TFC	30.37 ± 0.106	25.36 ± 0.502	14.32 ± 0.134	12.61 ± 0.360	31.61 ± 0.516	26.47 ± 0.431	14.5 ± 0.551	14.42 ± 0.480
The results were expressed as Mean $\pm$ Standard deviation.								

In terms of flavonoid content, the methanolic extracts also showed the highest TFC, with the methanolic leaf extract containing 30.375 ± 0.1060 mg QE/g dry weight and the flower extract containing 31.615 ± 0.5161 mg QE/g dry weight. Acetone, chloroform, and hexane extracts had lower TFC values: 25.365 ± 0.5020 , 14.325 ± 0.1343 , and 12.615 ± 0.3606 mg QE/g dry weight for leaves, and 26.475 ± 0.4313 , 14.5 ± 0.5515 , and 14.42 ± 0.4808 mg QE/g dry weight for flowers. These results are consistent with the data reported by Kapoor and Rani.[16]

Overall, the phenolic and flavonoid contents varied significantly across different extracts, with polar solvents like methanol and acetone containing higher concentrations of these bioactive compounds than non-polar solvents like hexane and chloroform. The detailed data on the TPC and TFC are instrumental for further studies aimed at isolating and identifying the specific bioactive compounds in *V. rosea*. The standard curve of gallic acid used for TPC determination is provided in Fig. 3.

GC-MS Analysis of extracts

The GC-MS analysis of the leaves and flowers of *V. rosea* using methanol (Tables 5 and 6), acetone, chloroform, and hexane extracts (**Tables S3-S8, refer to SI**) revealed a diverse array of chemical compounds with significant medicinal properties. Both plant parts exhibited a range of bioactive compounds beneficial to human health, including antibiotics, antimicrobials, antidiabetic agents, and anticancer agents. Notably, alkaloids such as vindoline, isovindolinone, 2,20-Cycloaspidospermidine-3-carboxylic acid, 6,7-didehydro-, methyl ester, (2 α ,3 α ,5 α ,12 α ,19 α ,20R)-, vincoline, and (+)-vincadifformine—renowned for their anticancer properties—were detected. These alkaloids were exclusively found in the methanolic and chloroform extracts.

Table 5
GC-MS analysis of methanolic extract of leaves of *V. rosea*

Peaks	Compound Name	Molecular Formula	Retention Time	Area %	m/z
1	Glycerol	C ₃ H ₈ O ₃	16.006	17.58	92
2	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C ₆ H ₈ O ₄	16.556	1.68	144
3	Sandalore	C ₁₄ H ₂₆ O	31.457	0.59	210
4	Dihydroactinidiolide	C ₁₁ H ₁₆ O ₂	32.574	0.43	180
5	1,2-Dimethyl-3,5-bis(1-methylethenyl) cyclohexane	C ₁₄ H ₂₄	33.151	0.09	192
6	Megastigmatrienone A	C ₁₃ H ₁₈ O	36.278	0.17	190
7	Caryophyllene acetate	C ₁₇ H ₂₈ O ₂	38.869	0.28	264
8	Myristic acid	C ₁₄ H ₂₈ O ₂	41.843	0.13	228
9	17-Oxo-4-propyl-3,4-seco-4-androsten-3-oic acid methyl ester	C ₂₃ H ₃₆ O ₃	42.027	0.23	360
10	2,6,10-Trimethylundecan-(5E)-2,5,9-Trien-4-one	C ₁₄ H ₂₂ O	42.239	0.15	206
11	5-(2-Butenyl)-17-oxo-4-nor-3,5-seco-5.alpha.-androstan-3-oic acid, methyl ester	C ₂₃ H ₃₆ O ₃	42.542	0.14	346
12	3H-3,10a-Methano-1,2-benzodioxocin-3-ol, octahydro-7,7-dimethyl-, (3.alpha.,6a.beta.,10a.beta.)-	C ₁₃ H ₂₂ O ₃	43.537	0.31	226
13	alpha-Cedrene epoxide	C ₁₅ H ₂₄ O	43.763	0.08	220
14	Phytol	C ₂₀ H ₄₀ O	43.876	0.69	296
15	hexahydrofarnesyl acetone	C ₁₈ H ₃₆ O	44.082	0.15	268
16	Neophytadiene	C ₂₀ H ₃₈	45.308	0.13	278
17	Inositol	C ₆ H ₁₂ O ₆	46.743	0.22	180
18	Methyl palmitate	C ₁₇ H ₃₄ O ₂	46.907	1.95	270
19	Butyl octyl phthalate	C ₂₀ H ₃₀ O ₄	47.804	0.31	334
20	Palmitic acid	C ₁₆ H ₃₂ O ₂	48.745	16.38	256
21	Methyl linoleate	C ₁₉ H ₃₄ O ₂	52.089	1.18	294

Compounds are categorized according to different classes.

Alcohol and derivatives (1,14,17,23,27,54); Aldehydes and ketones (2,4,10,11,25,28,29,53); Alkanes and hydrocarbons (5,16,31,34,39); Aromatic compounds and derivatives (3,12,13,32,33,36,42); Carboxylic acids and derivatives (8,9,20,26,30,35,18,21,22,24); Esters and lactones (7,27,55); Steroids and Terpenes (6,13,46,47,48,50,51,52,56); Miscellaneous (19,37,38,40,41,43,44,45,49)

Peaks	Compound Name	Molecular Formula	Retention Time	Area %	m/z
22	Methyl oleate	C ₁₉ H ₃₆ O ₂	52.319	3.88	296
23	Phytol	C ₂₀ H ₄₀ O	52.704	3.42	296
24	Methyl stearate	C ₁₉ H ₃₈ O ₂	53.181	0.4	298
25	(Z)-Tetradec-7-enal	C ₁₄ H ₂₆ O	54.093	19.61	210
26	Stearic acid	C ₁₈ H ₃₆ O ₂	54.707	1.44	284
27	4,8,12,16-Tetramethylheptadecan-4-olide	C ₂₁ H ₄₀ O ₂	59.582	0.27	324
28	7a-Methyl-1,2,3,6,7,7a-hexahydro-5H-inden-5-one	C ₁₀ H ₁₄ O	60.03	0.45	150
29	2-cyclohexen-1-one, 3-methyl-5-(1-methylethyl)-4-(2-methyl-1-propenyl)-	C ₁₄ H ₂₂ O	60.649	0.68	206
30	Hexanoic acid 2-ethyl-hexadecyl ester	C ₂₄ H ₄₈ O ₂	61.94	0.84	368
31	2-Methylhexacosane	C ₂₇ H ₅₆	62.409	0.17	380
32	Isoindoline	C ₂₁ H ₂₄ N ₂ O ₂	63.262	0.59	336
33	Isovindolinone	C ₂₁ H ₂₄ N ₂ O ₂	63.524	0.79	336
34	Dotriacontane	C ₃₂ H ₆₆	63.807	0.12	450
35	Hexanoic acid 2-ethyl-hexadecyl ester	C ₂₄ H ₄₈ O ₂	64.689	0.35	368
36	Coronaridine	C ₂₁ H ₂₆ N ₂ O ₂	65.019	0.57	338
37	cis-Cyclo(Leucyl-Tyrosyl)	C ₁₅ H ₂₀ N ₂ O ₃	65.55	0.16	276
38	cis-Cyclo(Leucyl-Tyrosyl)	C ₁₅ H ₂₀ N ₂ O ₃	65.832	0.36	276
39	Squalene	C ₃₀ H ₅₀	66.219	1.03	410
40	alpha-Tocospiro A	C ₂₉ H ₅₀ O ₄	66.56	0.3	462
41	alpha-Tocospiro-B	C ₂₉ H ₅₀ O ₄	66.804	0.41	462
42	Desmethoxyvindoline	C ₂₄ H ₃₀ N ₂ O ₅	68.134	1.24	426
43	Stigmast-5-en-3-ol, oleate	C ₄₇ H ₈₂ O ₂	69.851	0.67	679
44	Vitamin E	C ₂₉ H ₅₀ O ₂	70.541	1.64	430
45	Vindoline-d3	C ₂₅ H ₃₂ N ₂ O ₆	71.842	5.5	459

Compounds are categorized according to different classes.

Alcohol and derivatives (1,14,17,23,27,54); Aldehydes and ketones (2,4,10,11,25,28,29,53); Alkanes and hydrocarbons (5,16,31,34,39); Aromatic compounds and derivatives (3,12,13,32,33,36,42); Carboxylic acids and derivatives (8,9,20,26,30,35,18,21,22,24); Esters and lactones (7,27,55); Steroids and Terpenes (6,13,46,47,48,50,51,52,56); Miscellaneous (19,37,38,40,41,43,44,45,49)

Peaks	Compound Name	Molecular Formula	Retention Time	Area %	m/z
46	Campesterol	C ₂₈ H ₄₈ O	72.477	0.89	400
47	Stigmasterol	C ₂₉ H ₄₈ O	73.034	0.6	412
48	beta.-Sitosterol	C ₂₉ H ₅₀ O	74.443	1.91	414
49	5H-3,5a-Epoxy-naphth[2,1-c]oxepin,dodecahydro-3,8,8,11a tetramethyl-	C ₁₈ H ₃₀ O ₂	75.356	0.49	278
50	24-Norursa-3,12-diene	C ₂₉ H ₄₆	75.667	1.03	394
51	24-Norursa-3,12-diene	C ₂₉ H ₄₆	76.604	1.01	394
52	Olean-12-en-3-ol, acetate, (3beta)-	C ₃₂ H ₅₂ O ₂	77.641	0.69	468
53	γ-Sitostenone	C ₂₉ H ₄₈ O	77.852	0.27	412
54	alpha-Fernenol	C ₃₀ H ₅₀ O	78.139	0.51	426
55	Methyl Commate A	C ₃₂ H ₅₂ O ₄	79.121	3.57	470
56	beta-Simiarenol	C ₃₀ H ₅₀ O	79.631	1.25	426
				100	
Compounds are categorized according to different classes.					
Alcohol and derivatives (1,14,17,23,27,54); Aldehydes and ketones (2,4,10,11,25,28,29,53); Alkanes and hydrocarbons (5,16,31,34,39); Aromatic compounds and derivatives (3,12,13,32,33,36,42); Carboxylic acids and derivatives (8,9,20,26,30,35,18,21,22,24); Esters and lactones (7,27,55); Steroids and Terpenes (6,13,46,47,48,50,51,52,56); Miscellaneous (19,37,38,40,41,43,44,45,49)					

Table 6
GC-MS analysis of methanolic extract of flowers of *V. rosea*

Peaks	Compound Name	Molecular Formula	Retention Time	Area %	m/z
1	Hydroperoxide, 1-methylbutyl	C ₅ H ₁₂ O	3.24	1.21	104.14
2	1H-Pyrazole-1-carbothioamide, 3,5-dimethyl-	C ₆ H ₉ N ₃ S	3.69	4.98	155.22
3	Cyclooctaneacetic acid, 2-oxo-	C ₁₀ H ₁₆ O ₃	3.95	1.36	184.23
4	Isoxazolidine, 5-ethyl-2,4-dimethyl-, trans-	C ₇ H ₁₅ NO	4.75	0.9	129.20
5	N-(5-Fluoro-tricyclo[2.2.1.0*2,6*]hept-3-yl)-acetamide	C ₉ H ₁₂ FNO	5.25	6.51	169.01
6	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	C ₆ H ₈ O ₄	5.54	2.61	144.12
7	3-Amino-2-oxazolidinone	C ₃ H ₆ N ₂ O ₂	6.50	1.18	102.09
8	Unidentified	NA	6.67	0.05	NA
9	Acetamide, N-acetyl-N-(1-methylpropyl)-	C ₈ H ₁₅ NO ₂	7.10	1.91	157.21
10	2,3-Dimethyldecane	C ₁₂ H ₂₆	7.27	1.82	170.33
11	1-Fluorononane	C ₉ H ₁₉ F	7.73	1.02	146.25
12	Benzene, 1-isocyano-3-methyl-	C ₈ H ₇ N	7.88	2.8	117.15
13	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	C ₆ H ₈ O ₄	8.11	4.37	144.12
14	5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	9.23	8.77	126.11
15	Sucrose	C ₁₂ H ₂₂ O ₁₁	13.14	3.61	342.30
16	á-D-Glucopyranoside, methyl 3,6-anhydro-	C ₇ H ₁₂ O ₅	14.94	3.09	176.17
17	3-O-Methyl-d-glucose	C ₇ H ₁₄ O ₆	17.06	2.71	194.18
18	Myo-Inositol, 4-C-methyl-	C ₇ H ₁₄ O ₆	17.24	1.13	194.18
19	Decanoic acid, 2-methyl-	C ₁₁ H ₂₂ O ₂	17.55	2.81	186.29
20	7-Hexadecenoic acid, methyl ester, (Z)-	C ₁₇ H ₃₂ O ₂	19.26	3.54	268.4
21	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	C ₁₁ H ₁₆ O ₄	20.19	3.52	212.24
22	Heptadecanoic acid, heptadecyl ester	C ₃₅ H ₇₀ O ₂	20.35	0.93	522.9

Compounds are categorized according to different classes.

Hydroperoxides (1); Pyrazoles (2); cycloalkanes (3); Isoxazolidines (4); Tricyclic compounds (5); Pyranones (6.13); oxazolidinones (7); Amides (9); Alkanes (10,11,25); Isocyanides (12); Furfural derivatives (14); Sugar and Sugar derivatives (15, 16, 17,18); Fatty acids and derivatives (19,20,22,27,28); Bicyclic compounds (21); Ethers (23); Amines (24); Alkaloids (26)

Peaks	Compound Name	Molecular Formula	Retention Time	Area %	m/z
23	Cetyl glycidyl ether	C ₁₉ H ₃₈ O ₂	21.21	1.58	298.5
24	1-Aminononadecane, N-trifluoroacetyl-	C ₂₁ H ₄₀ F ₃ NO	23.35	1.22	379.54
25	2-Bromotetradecane	C ₁₄ H ₂₉ Br	23.28	31.16	277.28
26	2,20-Cycloaspidospermidine-3-carboxylic acid, 6,7-didehydro-, methyl ester, (2à,3à,5à,12à,19à,20R)-	C ₂₁ H ₂₆ N ₂ O ₂	25.42	2.26	338.18
27	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	C ₂₁ H ₄₀ O ₄	28.00	0.56	356.5
28	9-octadecenoic acid, 2,2,2-trifluoroethyl ester	C ₂₀ H ₃₅ F ₃ O ₂	28.70	2.42	364.5
Total				100	
Compounds are categorized according to different classes.					
Hydroperoxides (1); Pyrazoles (2); cycloalkanes (3); Isoxazolidines (4); Tricyclic compounds (5); Pyranones (6.13); oxazolidinones (7); Amides (9); Alkanes (10,11,25); Isocyanides (12); Furfural derivatives (14); Sugar and Sugar derivatives (15, 16, 17,18); Fatty acids and derivatives (19,20,22,27,28); Bicyclic compounds (21); Ethers (23); Amines (24); Alkaloids (26)					

The analysis also identified compounds with antimicrobial, antidiabetic, and anti-inflammatory activities, underscoring the therapeutic potential of *V. rosea*. The solvent choice significantly impacted extraction efficiency and the resultant compound profiles. Methanol extracts from both leaves and flowers revealed a broad spectrum of compounds. The leaves exhibited a high percentage of palmitic acid (16.38%) and (Z)-Tetradec-7-enal (19.61%), indicating a prominent presence of lipids and aldehydes. In contrast, the flowers' acetone extracts showed elevated levels of 2-bromotetradecane (31.16%) and diacetone alcohol (42.78%), highlighting a distinct chemical composition.

The variation in compound categories detected—such as alkanes, phenols, flavonoids, and halogenated compounds—emphasizes the critical role of solvent selection in isolating specific bioactive compounds. GC-MS has proven to be an indispensable tool in differentiating and identifying these compounds, which enhances our understanding of *V. rosea*'s medicinal properties and potential applications. The GC-MS chromatograms are presented in Fig. 4.

In comparing the chemical profiles across the methanol, acetone, chloroform, and hexane extracts from both leaves and flowers, distinct patterns and unique compounds emerged. The methanol extract of leaves (Table 5) exhibited a complex profile, including alcohols and their derivatives, aldehydes, ketones, carboxylic acids, steroids, alkaloids and terpenes, positioning it as a source of diverse bioactive substances. Conversely, the methanol extract of flowers (Table 6) revealed an intriguing mix of hydroperoxides, pyrazoles, cycloalkanes, and alkaloids, presenting a unique chemical fingerprint.

The acetone extract of leaves (Table S3) was characterized by significant quantities of alkanes, ethers, aldehydes, alcohols, and esters, offering a comprehensive profile of both lipophilic and polar compounds. In comparison, the acetone extract of flowers (Table S4) contained distinct classes, such as azomethanes, ketones, and fatty acids, with notable alkaloid presence, differentiating it from the leaf extract.

The chloroform extract of leaves (Table S5) was distinguished by its high concentration of halogenated compounds, esters, alcohols, alkanes, and carboxylic acids, indicating potential for unique therapeutic applications. The chloroform

extract of flowers (**Table S6**) shared these components but also included terpenoids and alkaloids, further enriching its chemical diversity.

Finally, the hexane extract of leaves (**Table S7**) featured a strong profile of alkanes, alcohols, esters, terpenes, and carboxylic acids, highlighting its diverse applications. The hexane extract of flowers (**Table S8**) was marked by its content of alcohols, carboxylic acids, esters, aromatic compounds, and thiol esters, which were absent in the leaf extract. Unique compounds identified included pyrazoles and isoxazolidines in the methanol extract of flowers; azomethanes and fatty acids in the acetone extract of flowers; sulfonyl chlorides, nitriles, and lactams in the chloroform extract of leaves; and chromenes and thiol esters in the hexane extracts of leaves and flowers, respectively. This comprehensive comparison highlights the rich and varied chemical landscapes of the different extracts, each contributing unique elements to their respective profiles.

Isolation of alkaloid from the leaves of *V. rosea*

Vinca rosea is renowned for its alkaloid content and their medicinal applications. In this study, vindoline (Fig. 5) was isolated from the leaves of *V. rosea* through a pH-adjusted process (Refer to SI for the whole process), yielding fine needle-like crystals with a melting point of 167°C and a yield of 0.08%. The presence of vindoline was confirmed by GC-MS analysis of the methanolic leaf extract (Table 5). Vindoline's high concentration in *V. rosea*, as reported by Kumar et al. [32] and Tiong et al.,[33] makes it valuable for commercial extraction and the production of other alkaloids. The purity of vindoline was verified using TLC, with an R_f value of 0.35 at 15% ethyl acetate in hexane.

The spectroscopic analysis further confirmed the structure of vindoline. The IR spectrum showed bands at ν_{max} : 3700 – 3500 cm⁻¹ (O-H stretching), 3500 – 3300 cm⁻¹ (N-H stretching), 2900 – 2800 cm⁻¹ (C-H stretching), 2360 – 2310 cm⁻¹ (R–N–C stretching), 1750 – 1650 cm⁻¹ (C = O stretching), 1500 – 1400 cm⁻¹ (C-C stretching), 1260 – 1250 cm⁻¹ (C-N stretching of aromatic amines), 1250 – 1000 cm⁻¹ (C-N stretching of aliphatic amines), and 1000 – 600 cm⁻¹ (= C-H bending). In the ¹H NMR spectrum, peaks were observed at δ 8.76 (1H, s, -OH), 7.04 (1H, d, J = 8.2 Hz, C9-H), 6.28 (1H, dd, J = 2.25 Hz, C10-H), 6.20 (1H, d, J = 2.2 Hz, C12-H), 5.81 (1H, ddd, J = 1.3, 3.45, 4.05 Hz, C14-H), 5.19 (1H, s, C17-H), 5.09 (1H, br-d, J = 10.15 Hz, C15-H), 3.71 (3Hs, s, 11-OMe), 3.65 (3Hs, s, 22-OMe), 3.55 (1H, s, C2-H), 3.40 (1H, dd, J = 4.65, 12.2 Hz, C3-H), 3.26 (1H, m, C5-H), 2.78 (1H, br-d, J = 16.3 Hz, C5-H), 2.61 (1H, m, C21-H), 2.57 (3Hs, s, NMe), 2.19 (2Hs, m, C6-H), 1.94 (3H, s, COMe), 1.46 (1H, m, C19-H), 0.93 (1H, m, C19-H), and 0.41 (3H, t, J = 7.3 Hz, C18-H). The ¹³C NMR spectrum displayed 25 signals at δ 171.54, 169.94, 160.44, 153.39, 129.83, 125.30, 124.31, 123.01, 104.48, 95.44, 82.74, 78.62, 75.77, 66.14, 54.98, 51.61, 51.07, 50.35, 43.60, 42.35, 37.96, 30.33, 20.63, and 7.45 ppm. The mass spectrum of vindoline showed a parent ion peak at m/z 188.102, with daughter ions at m/z 162.025, 194.154, 222.456, 338.285, 380.246, 398.212, 440.275, and 458.231. These spectral features closely match those reported for vindoline by Wen [34] and Huat,[35] confirming the compound's identity (Refer to SI for Spectra).

Antifungal activity

The antifungal activity of various extracts and isolated compounds from *V. rosea* leaves and flowers were evaluated against two wheat pathogens, *F. graminearum* and *B. sorokiniana*, using the poison food technique at different concentrations (125, 250, 500, and 1000 µg/ml). The effectiveness of these treatments was compared to the standard fungicide propiconazole (Tilt 25 EC), assessing ED₅₀ and ED₉₀ values, which represent the doses required to inhibit 50% and 90% of mycelial growth, respectively.

For *F. graminearum*, the methanolic extract of *V. rosea* leaves exhibited the highest antifungal activity with 99.05% inhibition at 1000 µg/ml, followed by acetone, chloroform, and hexane (**Table S9**). Acetone and chloroform extracts showed moderate inhibition, while hexane extract was less effective. Similarly, flower extracts against *F. graminearum* showed that the methanolic extract was highly effective, with 97.91% inhibition at 1000 µg/ml, followed by the acetone

(Fig. 6). Other extracts showed lesser activity. Vindoline demonstrated the highest inhibition against *F. graminearum* (99.77% at 1000 µg/ml), equivalent to the standard fungicide. The antifungal activity ranking was Tilt > Vindoline > Methanol extract > Acetone extract > Chloroform extract > Hexane extract. The vindoline and methanol extract of leaves and flowers had an ED₅₀ value of 68, 80, and 82 µg/ml, close to the standard Tilt (62.5 µg/ml), indicating its potential as an effective fungicide.

Against *B. sorokiniana*, the methanolic extract of *V. rosea* leaves showed high antifungal activity (98.01% at 1000 µg/ml), followed by the acetone and chloroform extracts which were moderately effective, while hexane extracts were less effective (Fig. 7). The methanol extract had an ED₅₀ value of 76 µg/ml, indicating strong antifungal potential. Similarly, flower extracts exhibited high antifungal activity against *B. sorokiniana*, with the methanolic extract showing 94.64% inhibition at 1000 µg/ml and ED₅₀ value of 90 µg/ml, demonstrating its potential efficacy. Vindoline is the most effective candidate against both fungi (**Table S10**). In comparison to the study,[36–37] which demonstrated the antifungal activity of the alcohol extract of *Curcuma longa* against eleven fungal species, with EC50 values (mg/mL) as follows: *Fusarium graminearum* (0.1088), *Fusarium chlamydosporum* (0.1742), *Alternaria alternata* (0.1888), *Fusarium tricinctum* (0.2547), *Sclerotinia sclerotiorum* (0.3135), *Botrytis cinerea* (0.3825), *Fusarium culmorum* (0.4229), *Rhizopus oryzae* (1.2086), *Cladosporium cladosporioides* (4.5176), *Fusarium oxysporum* (3.8833), and *Colletotrichum higginsianum* (5.0183), our study found that the methanolic extract of *V. rosea* leaves and flowers, as well as vindoline, exhibited superior antifungal activity compared to the reported values.[36–37] Specifically, the vindoline showed more effective inhibition against the tested fungal species, indicating a potentially higher potency of the methanolic extract and vindoline relative to the *Curcuma longa* extract.

The effectiveness of the methanolic and acetone extracts can be attributed to their high alkaloid content, which provides stronger inhibition against wheat fungi compared to the hexane extracts. The hexane extracts exhibited the least antifungal activity, primarily composed of alkanes, alcohols, esters, terpenes, and carboxylic acids. Alkaloids, due to their complex and structurally effective inhibition mechanisms, play a crucial role in this enhanced activity. The robust antifungal effect of the methanolic extract is likely linked to its substantial bioactive alkaloid content, including vindoline, which is also noted for its anticancer properties, along with phenols and flavonoids that have antimicrobial activities. Alkaloids disrupt fungal cell membranes, impairing cell function and inhibiting fungal growth. Similarly, lipophilic terpenoids can interfere with fungal cell membranes, hindering spore germination and mycelial growth.[36–39]

The statistical analysis revealed a positive correlation between antifungal activity and concentration, with the standard fungicide propiconazole (Tilt 25 EC) being more effective than all other test components. Tukey multiple range tests confirmed significant differences between the mean values of various test components. This study concluded that the plant extracts and vindoline from *V. rosea* showed promising antifungal activity, with vindoline being more effective at lower concentrations. The results show that vindoline has substantial potential as an alternative antifungal agent, presenting an exciting opportunity for developing natural fungicides from plant sources. The reason for its effectiveness is the presence of an indole ring with various targeting sites, which probably leads to breaking the chain of fungal growth by attacking the core of its nucleus.

CONCLUSION

In conclusion, the comprehensive analysis of *V. rosea* highlights its significant potential as a source of bioactive compounds with diverse therapeutic applications. The phytochemical investigation confirmed the presence of various alkaloids, phenols, flavonoids, and other essential compounds contributing to its medicinal properties. Notably, the methanolic extracts demonstrated the highest yield and richest content of bioactive constituents, underscoring the

effectiveness of polar solvents in extracting these compounds. The antifungal assays revealed the notable efficacy of *Vinca rosea* extracts against wheat fungi, particularly *F. graminearum* and *B. sorokiniana*. The methanolic extracts showed exceptional antifungal activity, with high inhibition rates and low ED₅₀ values, suggesting their potential use in agricultural applications to combat phytopathogenic fungi, which further improved with the treatment of vindoline, a well-known anticancer precursor drug component. This study advances our understanding of *V. rosea*'s phytochemical and antifungal properties and opens avenues for its application in developing natural and effective pharmaceutical and agricultural products. The remarkable efficacy of *V. rosea* extracts, particularly the methanolic ones, is attributed to potent bioactive compounds like alkaloids in higher concentrations, which showed significant antifungal activity comparable to standard fungicides. This explains the potential of *V. rosea* as a valuable resource for natural antifungal agents and other therapeutic applications, offering an exciting prospect for future research and development.

Declarations

Declaration of competing interest

The authors confirm that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Author Contribution

Parul Sharma: Searched and worked on the project, extracted the data, wrote the original draft, given final approval of the version to be published. Ramandeep Kaur: Conceptualization, supervision, drafting and revising the manuscript, and giving the final approval of the version to be published. Jaspal Kaur: Worked on getting the antifungal activity data, conceptualization, and final approval of the version to be published. Urvashi Bhardwaj: Reviewed and final approved the version to be published. All authors have read and approved the final version of the manuscript submitted for publication and are responsible for the final content.

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Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Das A, Sarkar S, Bhattacharyya S, Gantait S. Biotechnological advancements in *Catharanthus roseus* (L.) G. Don. Appl Microbiol Biotechnol. 2020;104:4811–4835. <https://doi.org/10.1007/s00253-020-10592-1>

2. Almagro L, Fernández-Pérez F, Pedreño MA. Indole alkaloids from *Catharanthus roseus*: Bioproduction and their effect on human health. *Molecules*. 2015;20:2973–3000. <https://doi.org/10.3390/molecules20022973>
3. Pandey SR, Mallavarapu GR, Naqvi AA, Yadav A, Rai SK, Srivastava S, et al. Volatile components of leaves and flowers of periwinkle *Catharanthus roseus* (L.) G. Don from New Delhi. *Flavour Fragr J*. 2006;21:427–430. <https://doi.org/10.1002/ffj.1606>
4. Balaabirami S, Patharajan S. In vitro antimicrobial and antifungal activity of *Catharanthus roseus* leaves extract important pathogenic organisms. *Int J Pharm Sci*. 2012;4:487–490.
5. Ibrahim M, Mehjabeen SS, Narsu ML. Pharmacological evaluation of *Catharanthus roseus*. *Int J Appl Pharm*. 2011;2:165–173.
6. Prajakta JP, Ghosh JS. Antimicrobial activity of *Catharanthus roseus*: A detailed study. *Br J Pharmacol Toxicol*. 2010;1:40–44.
7. Sharma P, Singla N, Kaur R, Bhardwaj U. A review on phytochemical constituents and pharmacological properties of *Catharanthus roseus* (L.) G. Don. *J Med Plants Stud*. 2024a;12(3):131–156. <https://doi.org/10.22271/plants.2024.v12.i3b.1675>
8. Sharma P, Kaur R, Bhardwaj U, Kaur J. Chemical composition and antifungal potential of *Vinca rosea* leaf essential oil and extracts from Northern India. *Cogent Food Agric*. 2024b;10(1). <https://doi.org/10.1080/23311932.2024.2382317>
9. Sharma P, Kaur R. Comprehensive analysis and therapeutic potential of ginger essential oil. *J Pharmacogn Phytochem*. 2024c;13(3):420–425. <https://doi.org/10.22271/phyto.2024.v13.i3e.14984>
10. Barkat A, Mujeeb M. The comparative study of *Catharanthus roseus* extract and extract loaded chitosan nanoparticles in alloxan induced diabetics rats. *Int J Biol Res*. 2013;4:670–678. <https://doi.org/10.7439/ijbr.v4i12.412>
11. Agarwal S, Chettri N, Bisoyi S, Tazeen A, Vedamurthy AB, Krishna V, et al. Evaluation of in-vitro anthelmintic activity of *Catharanthus roseus* extract. *Int J Pharm Sci Drug Res*. 2011;3(3):211–213.
12. Rajput MS, Nair V, Chauhan A, Jawanjal H, Dange V. Evaluation of antidiarrheal activity of aerial parts of *Vinca major* in experimental animals. *Middle-East J Sci Res*. 2011;7:784–788.
13. Daniel M. *Medicinal Plants: Chemistry and Properties*. Science Publishers; 2006.
14. Renault JH, Nuzillard JM, Crouerour JL, Thepenier P, Zeches-Hanrot M, Olivier ML. Isolation of indole alkaloids from *Catharanthus roseus* by centrifugal partition chromatography in the pH-zone refining mode. *J Chromatogr A*. 1999;849:421–431. [https://doi.org/10.1016/S0021-9673\(99\)00495-1](https://doi.org/10.1016/S0021-9673(99)00495-1)
15. Hisiger S, Jolicoeur M. Analysis of *Catharanthus roseus* alkaloids by HPLC. *Phytochem Rev*. 2007;6:207–234. <https://doi.org/10.1007/s11101-006-9027-8>
16. Kapoor M, Rani J. Qualitative and quantitative analysis of phytochemicals by GC-MS and antioxidant activity of *Catharanthus roseus* (L.) G. Don. *J Pharmacogn Phytochem*. 2019;8:378–385.
17. Brun G, Bessière JM, Dijoux-Franca MG, David B, Mariotte AM. Volatile components of *Catharanthus roseus* (L.) G. Don (Apocyanaceae). *Flavour Fragr J*. 2001;16:116–119. <https://doi.org/10.1002/ffj.956>
18. Lawal OA, Ogunwande IA, Ibiroga AE, Opoku AR. Chemical constituents of essential oils from *Catharanthus roseus* (L.) G. Don grown in Nigeria. *J Essent Oil-Bear Plants*. 2015;18:57–63. <https://doi.org/10.1080/0972060X.2014.983435>
19. Sharma M, Chahal KK, Kaur R, Singh R, Kataria D. Antifungal potential and structure activity relationship of carrot seed constituents. *J Food Biochem*. 2019;43(1):1–9. <https://doi.org/10.1111/jfbc.12613>

20. Gupta SK, Tripathi SC. Fungitoxic activity of *Solanum torvum* against *Fusarium sacchari*. Plant Prot Sci. 2011;47:83–91. <https://doi.org/10.17221/20/2010-PPS>
21. Beyer M, Aumann J. Effects of *Fusarium* infection on the amino acid composition of winter wheat grains. Food Chem. 2008;111:750–754. <https://doi.org/10.1016/j.foodchem.2008.04.033>
22. Kumar P, Rai RC. Estimation of yield loss at different disease levels of spot blotch of wheat in Bihar. Int J Chem Stud. 2018;6:57–58.
23. Adhikari P, Pandey A, Agnihotri V, Pande V. Selection of solvent and extraction method for determination of antimicrobial potential of *Taxus wallichiana* Zucc. Res Pharm. 2018;8:1–9.
24. Bai G, Shaner G. Management and resistance in wheat and barley to *Fusarium* head blight. Annu Rev Phytopathol. 2004;42:135–161. <https://doi.org/10.1146/annurev.phyto.42.040803.140340>
25. De Wolf ED, Madden LV, Lipps PE. Risk assessment models for wheat *Fusarium* head blight epidemics based on within-season weather data. Phytopathology. 2003;93:428–435. <https://doi.org/10.1094/PHYTO.2003.93.4.428>
26. Aziz S, Saha K, Sultana N, Uddin A, Amin R, Al-Mansur. Comparative studies on proximate analysis and amino acid composition of leaf and flowers proteins conducted on medicinal plant, *Catharanthus roseus*, available in Bangladesh. World J Pharm Res. 2015;4(1):296–309.
27. Esther AE, Maureen CO, Ruffina AN, Ann MO, Chisom IF. Preliminary studies on the phytochemical and proximate composition of *Catharanthus roseus* (Linn). Int J Bot Stud. 2016;1(3):8–10.
28. Sahito SR, Kazi TG, Kazi GH, Jakhrani MA, Shaikh MS. Trace elements in two varieties of indigenous medicinal plant *Catharanthus roseus* (*Vinca rosea*). J Med Sci. 2001;1:74–77.
29. Srivastava P, Singh M, Devi G, Chaturvedi R. Herbal medicine and biotechnology for the benefit of human health. *Anim Biotechnol*. 2014;563–575. In: *Models in Discovery and Translation*. Academic Press, United States.
30. Goswami S. Preliminary phytochemical screening and standardisation of leaves of *Catharanthus roseus* (L.) G. Don. Indian J Res Pharm Biotechnol. 2013;1(1):24.
31. Masih N, Singh B. Phytochemical screening of some plants used in herbal based cosmetics preparations. In: Khemani L, Srivastava M, Srivastava S, editors. *Chemistry of Phytopotentials: Health, Energy and Environmental Perspectives*. Springer, Berlin, Heidelberg; 2012. p. 111–112.
32. Kumar S, Singh A, Kumar B, Singh B, Bahadur L, Lal M. Simultaneous quantitative determination of bioactive terpene indole alkaloids in ethanolic extracts of *Catharanthus roseus* (L.) G. Don by ultra-high performance liquid chromatography–tandem mass spectrometry. J Pharm Biomed Anal. 2018;151:32–41. <https://doi.org/10.1016/j.jpba.2018.01.055>
33. Tiong SH, Looi CY, Hazni H, Arya A, Paydar M, Wong WF, Cheah SC, Mustafa MR, Awang K. Antidiabetic and antioxidant properties of alkaloids from *Catharanthus roseus* (L.) G. Don. Molecules. 2013;18(8):9770–9784. <https://doi.org/10.3390/molecules18089770>
34. Wen B. Studies on the biosynthetic relationship between leucine and the indole alkaloids [dissertation]. Ottawa, ON: Carleton University; 1977.
35. Huat TS. Alkaloids of *Catharanthus roseus* and their hypoglycemic activity [master's thesis]. Kuala Lumpur: University of Malaya; 2014.
36. Chen C, Long L, Zhang F, Chen Q, Chen C, Yu X, Long Z. Antifungal activity, main active components, and mechanism of *Curcuma longa* extract against *Fusarium graminearum*. PLoS One. 2018;13(3) <https://doi.org/10.1371/journal.pone.0194284>
37. Hashmi LS, Hossain MA, Weli AM, Al-Riyami Q, Al-Sabahi JN. Gas chromatography-mass spectrometry analysis of different organic crude extracts from the local medicinal plant of *Thymus vulgaris* L. Asian Pac J Trop Biomed. 2013;3(1):69–73. [https://doi.org/10.1016/S2221-1691\(13\)60026-X](https://doi.org/10.1016/S2221-1691(13)60026-X)

38. Miron D, Battisti F, Silva FK, Lana AD, Pippi B, Casanova B, Gnoatto S, Fuentefria A, Mayorga P, Schapoval EES. Antifungal activity and mechanism of action of monoterpenes against dermatophytes and yeasts. *Rev Bras Farmacogn*. 2014;24(6):660–667. <https://doi.org/10.1016/j.bjp.2014.10.004>
39. Tyagi AK, Malik A. Liquid and vapour-phase antifungal activities of selected essential oils against *Candida albicans*: Microscopic observations and chemical characterization of *Cymbopogon citratus*. *BMC Complement Altern Med*. 2010;10(1):65. <https://doi.org/10.1186/1472-6882-10-65>

Figures

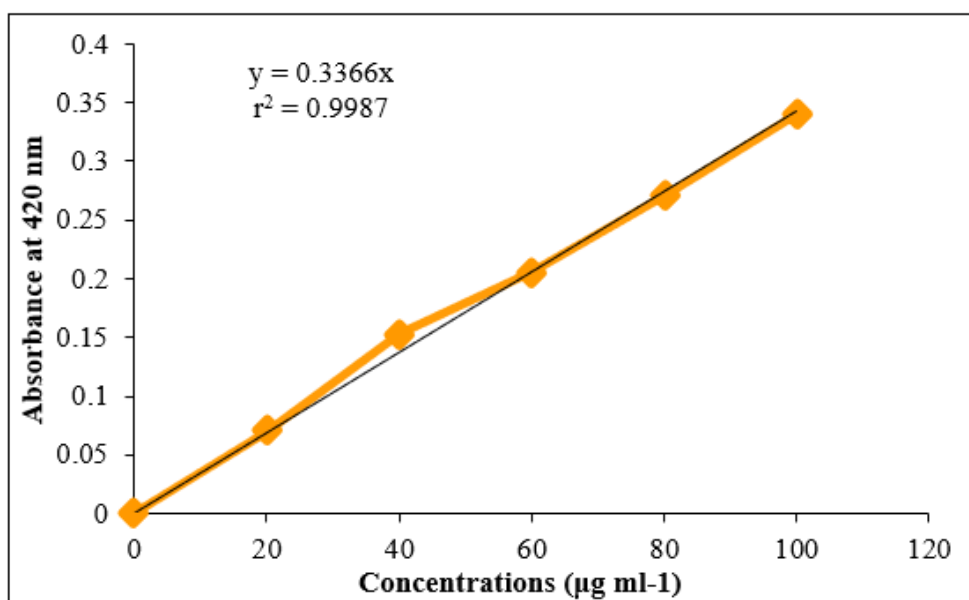


Figure 1

Standard curve of tannin

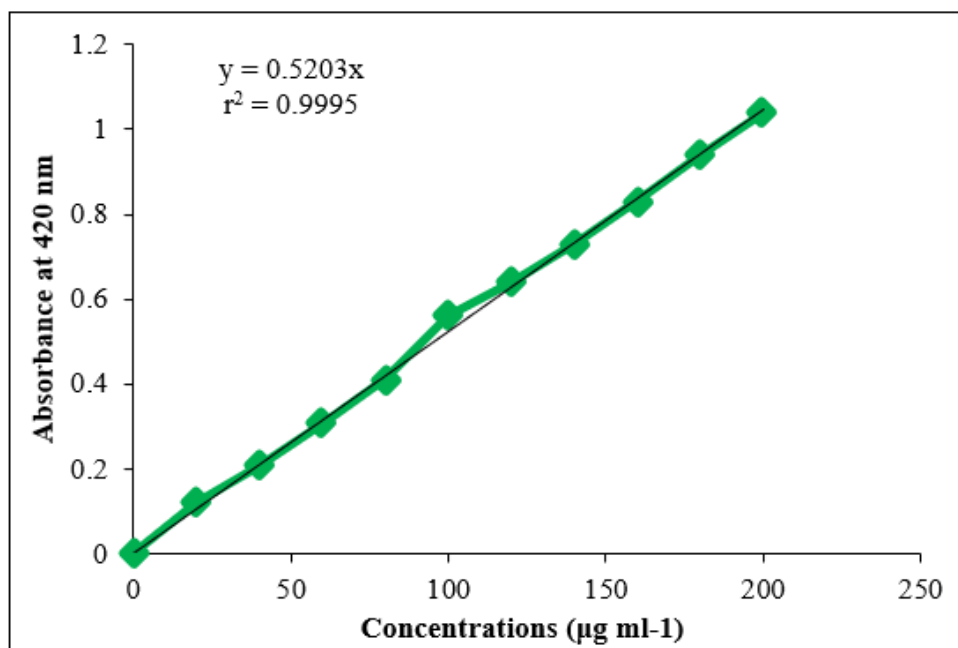


Figure 2

Standard curve of quercetin

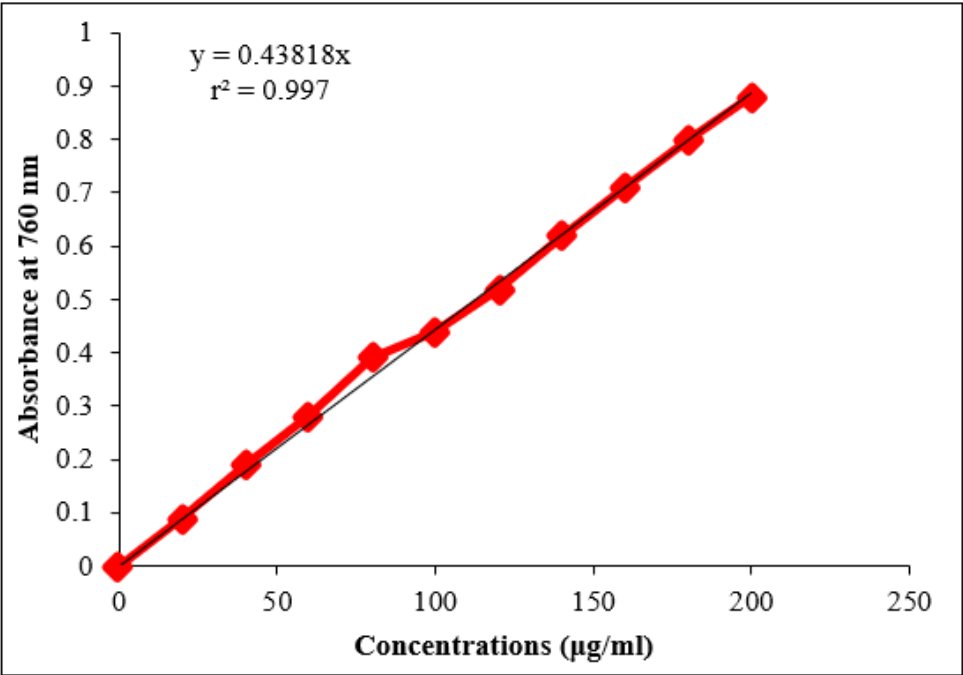


Figure 3

Standard curve of gallic acid

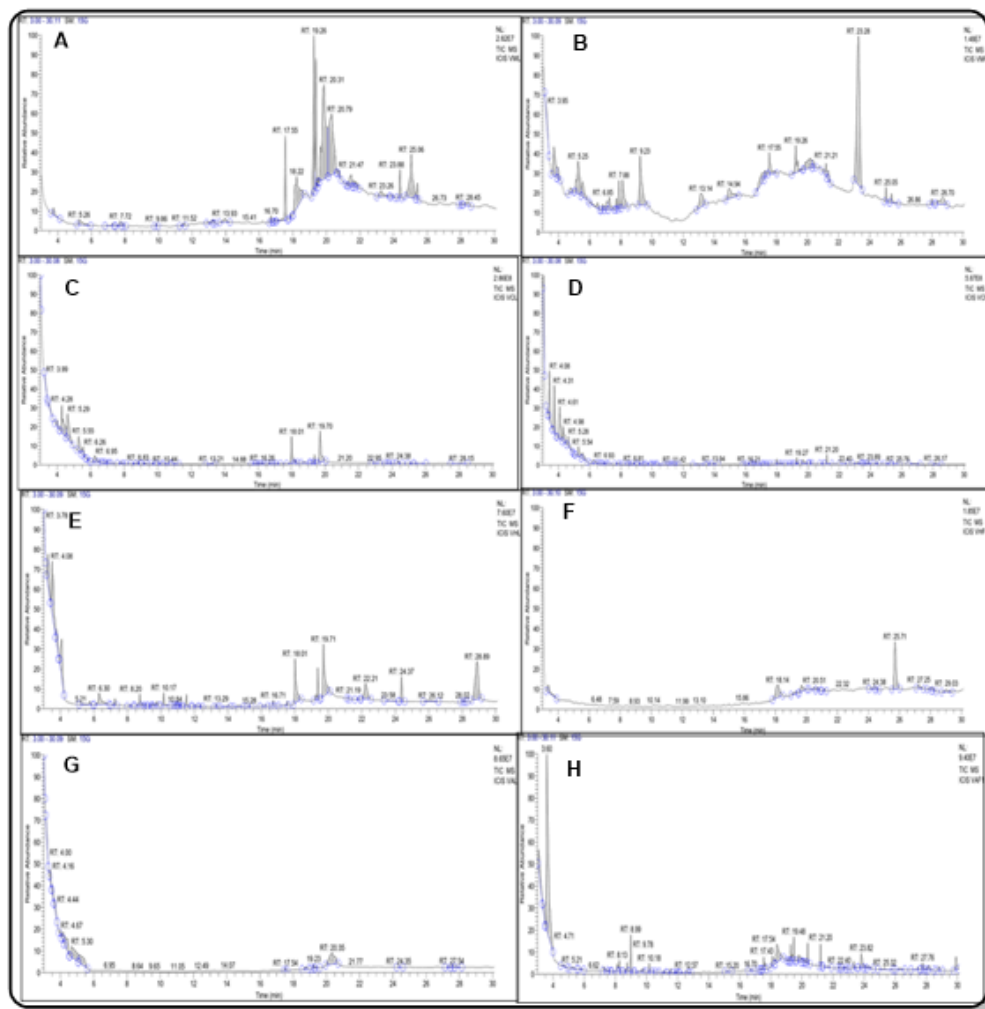


Figure 4

GC-MS chromatogram of various extracts obtained from the leaves and flowers of *V. rosea*

A: Leaves methanol extract; B: Flower methanol extract; C: Leaves acetone extract; D: Flower acetone extract; E: Leaves chloroform extract; F: Flower chloroform extract; G: Leaves hexane extract; H: Flower hexane extract

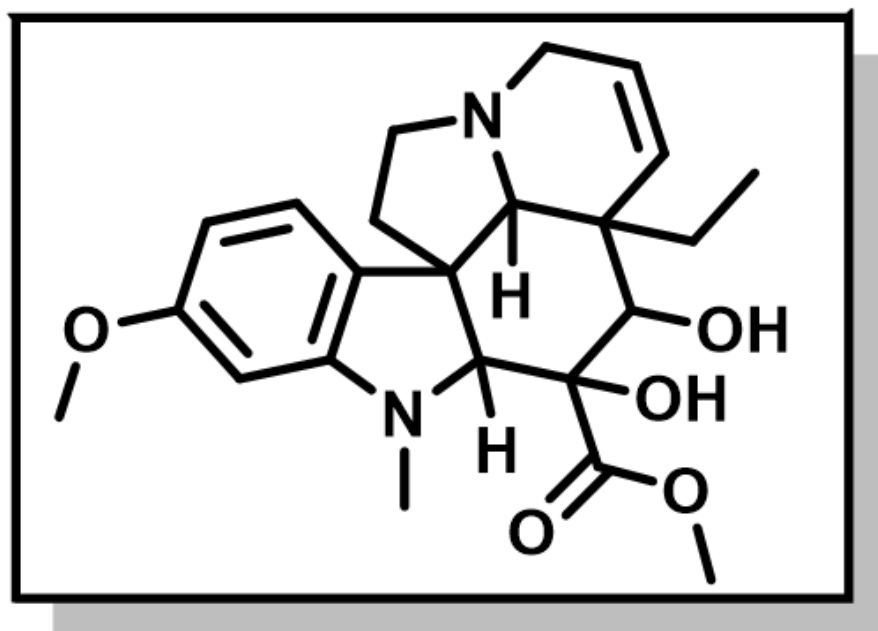


Figure 5

Structure of vindoline isolated from the leaves of *V. rosea*

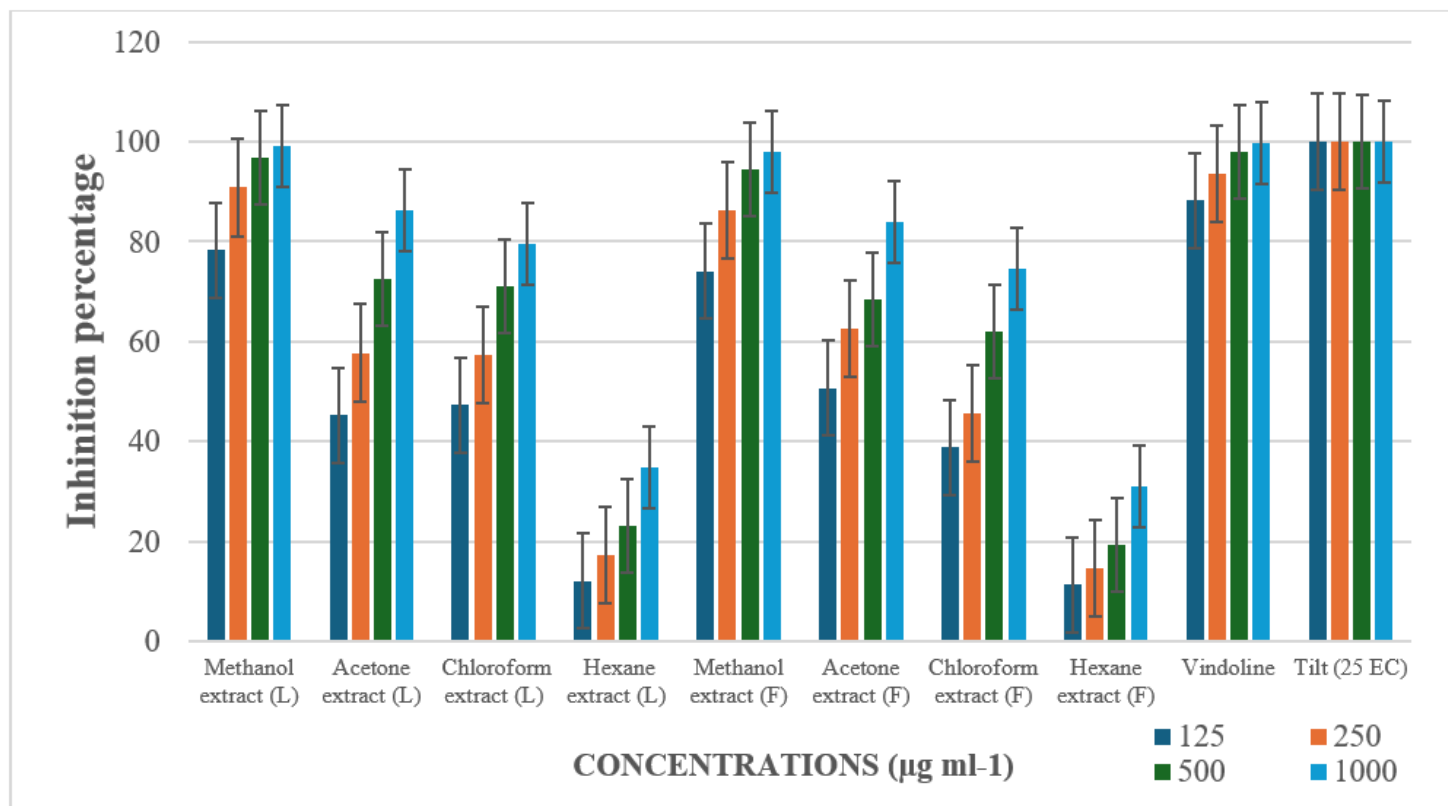


Figure 6

Percent mycelial growth inhibition of different treatments against *F. graminearum*

(L): Leaves; (F): Flower

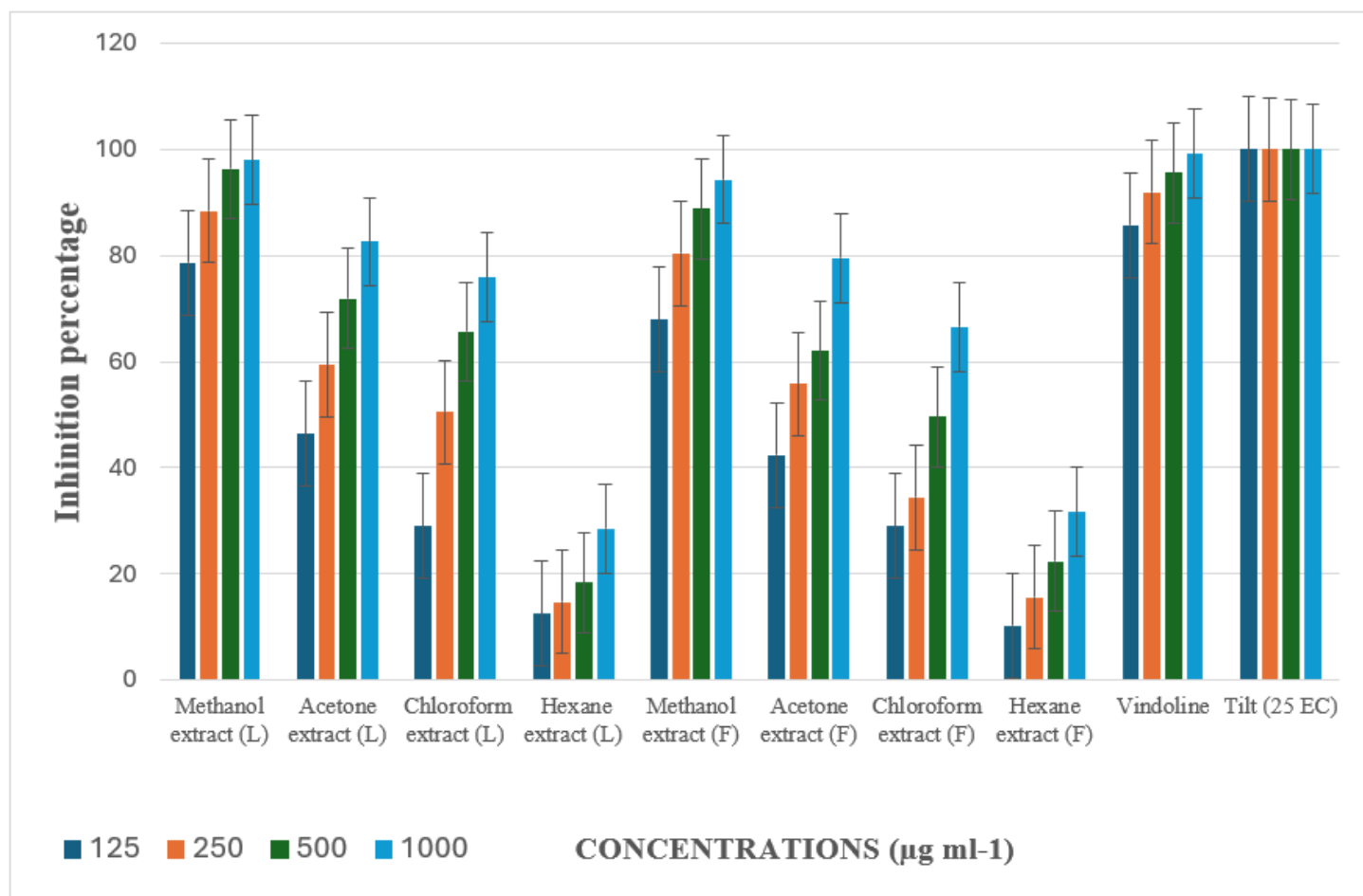


Figure 7

Percent mycelial growth inhibition of different treatments against *B. sorokiniana*

(L): Leaves; (F): Flower

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