

Deep Eutectic Solvents as Eco-Friendly Agents for Unlocking Antioxidants from *Catharanthus roseus*

Parul Sharma

Oklahoma State University

Ramandeep Kaur

ramansaini@pau.edu

Punjab Agricultural University

Avjot Kaur

Punjab Agricultural University

Research Article

Keywords: Vinca rosea, DPPH, Hydroxyl Radical Scavenging Activity, Sadabahar, DES solvent

Posted Date: May 19th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-6388547/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

This study explores the antioxidant potential of deep eutectic solvent (DES) extracts from *Catharanthus roseus*, also known as Madagascar periwinkle. DESs, noted for their biodegradability, low toxicity, and cost-effectiveness, offer a promising green alternative for bioactive compound extraction. Evaluating the antioxidant activity of *C. roseus* leaves using DESs advances natural product chemistry by highlighting their efficiency. Antioxidant activity was measured with DPPH and hydroxyl radical scavenging assays, showing that DES extracts exhibited significant antioxidant effects. Among the solvents tested, methanol showed the highest antioxidant activity, with ChCl Acid DES closely following. These results underscore the effectiveness of DESs in extracting potent natural antioxidants from *C. roseus*, presenting a sustainable method for developing therapeutic agents against oxidative stress-related diseases. This study highlights DESs' potential in improving extraction efficiency and promoting green chemistry practices in natural product extraction.

1. Introduction

In recent years, the principles of green and sustainable chemistry have emphasized the need to replace hazardous solvents with environmentally friendly alternatives across all scientific fields. One such alternative is deep eutectic solvents (DESs), a class of sustainable solvents that have garnered significant attention for their versatility and eco-friendliness. DESs are defined as binary or ternary systems comprising a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD), which interact to form a eutectic mixture with a melting point significantly lower than that of their individual components. Common HBAs include quaternary ammonium and phosphonium halide salts, while typical HBDs encompass amides, alcohols, carbohydrates, and organic acids.^{1–5} DESs are biodegradable, cost-effective, and simple to synthesize, making them a promising alternative to conventional organic solvents. Their minimal toxicity and sustainability have led to their widespread adoption in various fields, particularly in natural product extraction.^{1,2} Traditional extraction methods for plant-based compounds often use hazardous organic solvents, which pose environmental risks and yield low efficiency. In contrast, DESs offer a greener alternative, efficiently extracting compounds like flavonoids and phenolic acids with less environmental impact. The potential of DESs in enhancing the extraction efficiency of bioactive compounds is a reason for optimism in the field of natural product chemistry and green chemistry.^{6–10}

Catharanthus roseus (*Vinca rosea*), known for its anticancer properties, contains alkaloids such as vincristine, vinblastine, and vindoline. This dicotyledonous angiosperm is particularly valued for its anticancer properties. Beyond its role in cancer treatment, *C. roseus* exhibits a diverse range of pharmacological activities, including antifungal, antibacterial, antimicrobial, antioxidant, anthelmintic, antidiarrheal, and antidiabetic effects.^{11–20} These therapeutic properties stem from the plant's rich composition of bioactive compounds, including alkaloids, flavonoids, terpenes, phenols, amino acids, and volatile compounds.^{21–23}

Among these, alkaloids are the most abundant and pharmacologically active constituents of *C. roseus*, with over 400 identified alkaloids contributing to applications in pharmaceuticals, food additives, pesticides, fragrances, and flavoring agents. Notably, the two terpene indole alkaloids, vinblastine and vincristine, have been commercialized as potent anticancer drugs.^{24–30}

However, traditional extraction of these alkaloids relies on toxic solvents. DESs provide a sustainable option for this extraction, though research on their use for *C. roseus* is still limited.³ Following the discovery of DES, extracting various natural compounds, including flavonoids and phenols from different plant species and herbal medicines, has been reported.^{4,5,31–33} A few studies have focused on extracting anthocyanins from *C. roseus* using DES, with flower petals used to extract anthocyanins (cyanidins) using a lactic acid-glucose DES.⁶ Antioxidants are crucial for neutralizing free radicals, which are unstable molecules that can cause oxidative stress and contribute to various diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders. This study aims to evaluate the antioxidant properties of *C. roseus* extracts using DESs, exploring their potential health benefits as natural therapeutic agents. Plant antioxidants are increasingly in demand for their ability to neutralize free radicals and mitigate diseases like cancer and cardiovascular disorders without the side effects of synthetic compounds.

2. Experimental

Plant material

Catharanthus roseus 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. (Ref No. V1265780) 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 according to 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

Materials, Reagents, and Equipment

Catharanthus roseus plants were collected from Punjab Agricultural University, Ludhiana, Punjab, India. The leaves were separated from the twigs, shade-dried, and then powdered using a pestle and mortar. The powdered samples were stored in the dark at room temperature until further use. The chemicals used for the preparation of DES included choline chloride (Loba Chemie Pvt. Ltd.), citric acid (Loba Chemie Pvt. Ltd.), oxalic acid (Loba Chemie Pvt. Ltd.), urea (Loba Chemie Pvt. Ltd.), 1-naphthylamine (Central Drug House Pvt. Ltd.), glycerol (Sarabhai R Chemicals), ethylene glycol (Sarabhai R Chemicals), glucose (Loba Chemie Pvt. Ltd.), and sucrose (Loba Chemie Pvt. Ltd.), all obtained from Ludhiana, Punjab. The standards vinblastine sulfate and vincristine sulfate were obtained from United Biotech Pvt. Ltd. (UBPL), India. The equipment used during the experiment included a magnetic stirrer (Sonar), an oven (The Bharat Instruments & Chemicals), a sonicator (SB-5200 DTDN), and a UV-visible spectrophotometer (Shimadzu UV-1800).

DES Preparation

Eight deep eutectic solvents were prepared using choline chloride (ChCl) as the hydrogen bond acceptor (HBA). In specific molar ratios, ChCl was mixed with various hydrogen bond donors (HBDs) such as acids, alcohols, amines, and carbohydrates. The components were placed in a round bottom flask and heated to 80°C while constantly stirring with a magnetic stirrer until a homogeneous liquid formed. The resulting clear liquid indicated the formation of a particular DES from its parent compounds.^{1,7} The components, molar ratios, and preparation times for all DES are presented in Table 1. (Refer to Supporting Information for complete detail). The rest of the parameters for the analysis of the DES solvent, like physical parameters, standard curve, and qualitative data, have been mentioned in SI for reference. (Table S1-S11; Figure S1-S5 from Supporting Information).

Table 1 Composition and molar ratio of various deep eutectic solvents			
Category	Deep Eutectic Solvents	Molar Ratio	Preparation Time (min)
	HBA: HBD		
Acids	ChCl: Citric Acid	1:1	360
	ChCl: Oxalic Acid	1:1	20
Amines	ChCl: Urea	1:2	60
	ChCl: 1-Naphthylamine	1:2	60
Alcohols	ChCl: Glycerol	1:1	70
	ChCl: Ethylene Glycol	1:2	70
Sugars	ChCl: Glucose	1:1	240
	ChCl: Sucrose	1:1	290
ChCl: Choline Chloride			

Ultrasonic-Assisted Extraction

The extracts of *C. roseus* leaves were prepared using an ultrasound-assisted extraction technique with all the DES solvents. First, 50 mg of the dried, powdered leaves were weighed and transferred separately to 25 ml flasks. Then, 10 ml of DES was added to each flask and thoroughly mixed. The flasks were placed in an ultrasonic water bath at room temperature for approximately 40 minutes at a frequency of 50 kHz. The contents were then filtered to obtain the DES extracts of *C. roseus* leaves.^{1,7,9,10,11,35–37}(Refer to Supporting Information for complete detail)

Phytochemical Screening and Quantitative analysis

The different DES extracts of *C. roseus* leaves were analyzed for the presence of various bioactive compounds known to be present in the plant. Qualitative screening of these extracts was conducted following established procedures, as referenced in the literature. ^(3,12,23,28,) The study focused on detecting a wide range of bioactive constituents, including alkaloids, amino acids, flavonoids, glycosides, phenols, saponins, tannins, terpenoids, and cardiac glycosides. The methanol extract was also subjected to the same qualitative analysis for comparison. Detailed methodologies for these analyses are provided in Supporting Information.

Additionally, quantitative assessments of flavonoids and tannins were performed to determine their concentrations in the DES extracts. This analysis aimed to provide a comparative evaluation of the abundance of these bioactive compounds, further supporting their presence and potential contribution to the plant's medicinal properties.

Quantification of Flavonoid and

Antioxidant Activity Assay

The antioxidant activity was assessed using DPPH and hydroxyl radical scavenging assays.

DPPH Radical Scavenging Activity

An aliquot of 3 ml of 0.004% DPPH solution in ethanol and 0.1 ml of plant extract at various concentrations were mixed and incubated at 37°C for 30 minutes. The absorbance of the test mixture was read at 517 nm. The percentage of inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using the formula:^{13–16}

Percentage inhibition = $A_0 - A_1 / A_0 \times 100$ Where A_0 = Absorbance of the control; A_1 = Absorbance of the plant extract/ standard.

By implementing these green chemistry approaches, we aim to provide an eco-friendly and efficient method for extracting valuable compounds from *C. roseus*, thereby contributing to sustainable practices in natural product chemistry.

Hydroxyl Radical Scavenging Activity

This assay was based on the benzoic acid hydroxylation method. Hydroxyl radicals were generated in reaction mixtures containing phosphate buffer. In a screw-capped tube, 0.2 ml of sodium benzoate (10 mM), 0.2 ml of FeSO₄·7 H₂O (10 mM), and 0.2 ml of EDTA (10 mM) were added. Then the sample solution and a phosphate buffer (pH 7.4, 0.1 M) were added to give a total volume of 1.8 ml. Finally, 0.2 ml of H₂O₂ solution (10 mM) was added. The reaction mixture was then incubated at 37°C for 2 hours. Thereafter, the fluorescence was measured at 407 nm emission (Em) and excitation (Ex) at 305 nm. The spectrofluorometric changes were used to detect the damage by the hydroxyl radical.^{17–21,4}

GC-MS

The chemical composition of *Catharanthus roseus* ChI: Citric acid extract was examined using a Shimadzu QP2010 Plus GC-MS system. The analysis entailed injecting the sample in single-injection mode while keeping the split valve closed for the first minute to ensure optimal sample introduction, with the injector temperature set to 280°C. Helium gas was utilized as the carrier, maintaining a steady pressure of 69 kPa. The components of the extracts were separated using an Rtx-5 MS capillary column, which measured 30 meters in length, had an internal diameter of 20 mm, and a film thickness of 0.25 µm. The oven's temperature program started at 50°C for two minutes, then gradually increased to 180°C at a rate of 3°C per minute, and finally rose to 200°C at 10°C per minute. During mass spectrometry, electron ionization was performed at 70 eV, with an interface temperature of 200°C, and scanning was done over a mass range from 40 to 600 amu. The samples were injected in split mode (120:1 ratio) and diluted in methanol (1/100 v/v). The chemical constituents were quantified by normalizing the peak areas observed in the chromatogram.^{3,12,23,28}

The GC-MS analysis of extract revealed several bioactive alkaloids and flavonoids (Table 4). Key alkaloids identified include isoindoline, coronaridine, Desmethoxyvindoline, and vindoline. These compounds are noteworthy because vindoline is a commercially significant anti-cancer drug precursor synthesizing various pharmaceutical agents. Also showed the presence of phenols and flavonoids in large concentrations.

3. Results and Discussion

Phytochemical Screening

Phytochemical analysis of *Catharanthus roseus* extracts revealed diverse bioactive compounds, including flavonoids, tannins, saponins, terpenoids, carbohydrates, and phenolic compounds. The ChCI: citric acid and methanol extracts contained the highest number of these phytochemicals (Table 2), with a significantly higher concentration of bioactive constituents compared to other extracts. This abundance underscores the medicinal potential of these extracts and aids in identifying the key components that may contribute to their antioxidant activity, thereby supporting their traditional use. Based on these findings, four deep eutectic solvents (DES) and methanol were selected for further study to evaluate their antioxidant properties and overall effectiveness.^{22–30,40–43}

Table 2
Phytochemical analysis of plant extracts

Extracts	Chemical Constituents									
	Alkaloids	Flavonoids	Terpenoids	Phenols	Tannins	Saponins	Glycosides	Amino acids	Carbohydrates	Cardiac Glycosides
ChCl: Urea	+	+	-	-	+	-	-	+	-	-
ChCl: CA	+	+	+	+	+	+	+	+	+	+
ChCl: OA	+	+	+	+	-	-	+	+	-	+
ChCl: Gly	+	+	+	+	+	-	+	-	-	-
ChCl: EG	+	+	+	+	-	-	+	-	-	-
ChCl: Glu	+	+	-	-	-	-	+	+	+	-
ChCl: Suc	+	-	+	-	-	-	+	-	+	-
ChCl: 1-Napht	+	-	-	+	-	-	-	-	-	-
Methanol	+	+	+	+	+	+	+	+	+	+
+: present; -: Absent										

Antioxidant Activity

The antioxidant activity of *C. roseus* extracts was assessed using two standard assays: the DPPH radical scavenging assay and the hydroxyl radical scavenging assay. These assays measure the extracts' ability to neutralize free radicals, thereby providing an indication of their antioxidant potential.^{5,14,15,4, 34}

DPPH Radical Scavenging Activity

The DPPH assay is a widely used method for evaluating plant extracts' free radical scavenging ability. In this study, the DPPH assay revealed that *C. roseus* extracts possess strong antioxidant activity. Among the extracts tested, the methanol extract exhibited the highest DPPH scavenging activity, with an IC₅₀ value of 0.03 mg/ml, closely followed by the ChCl: Citric acid extract, which had an IC₅₀ value of 0.04 mg/ml. These results are comparable to the standard antioxidant ascorbic acid, which had an IC₅₀ value of 0.02 mg/ml (Table 3). The potent antioxidant activity observed in the methanol and ChCl: CA extracts can be attributed to their high phenolic compounds and flavonoid content. These compounds are effective hydrogen donors and can stabilize free radicals by forming resonance-stabilized phenoxyl radicals. These extracts' strong DPPH scavenging activity suggests their potential as natural antioxidants that could be used in therapeutic applications to mitigate oxidative stress-related conditions.^{13,31,38, 39}

Table 3
DPPH free radical percentage inhibition (%) of DES leaves extract of *C. roseus* at various concentrations

Leaves Extract	Concentrations (mg ml ⁻¹)					TUKEY TEST MEAN	IC ₅₀
	0.02	0.04	0.06	0.08	0.1		
ChCl:Citric Acid	41.7 ± 1.5	52.4 ± 0.4	61.6 ± 0.4	65.2 ± 0.5	70.3 ± 0.5	58.27 ^b	0.04
ChCl:Urea	12.1 ± 0.3	14.4 ± 0.4	17.2 ± 0.4	18.6 ± 0.3	20.7 ± 0.4	16.64 ^e	-
ChCl:Glycerol	25.5 ± 0.6	29.2 ± 0.5	34.8 ± 1.9	41.2 ± 0.5	49.7 ± 0.4	36.07 ^c	0.1
ChCl:Glucose	17.1 ± 0.7	20.3 ± 0.5	25.0 ± 0.8	29.7 ± 1.2	34.4 ± 0.7	25.26 ^d	-
Methanol	45.6 ± 0.3	56.8 ± 1.2	63.5 ± 0.7	68.2 ± 1.4	73.4 ± 0.8	61.52 ^b	0.03
Ascorbic acid (Standard)	49.5 ± 0.3	66.0 ± 0.4	70.7 ± 1.5	73.7 ± 0.5	79.1 ± 0.4	67.82 ^a	0.02
IC ₅₀ signifies concentrations (mg ml ⁻¹) for a 50% inhibition.							
Experiments were replicated three times and represented as mean ± standard deviation.							
The mean values having the same superscript are non-significant (p > 0.05) as per the Tukey multiple range test.							

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging assay further corroborated the antioxidant potential of *C. roseus* extracts. The results showed a similar trend to the DPPH assay, with the methanol and ChCl: CA extracts again displaying the highest scavenging activities, both with an IC₅₀ value of 0.05 mg/ml. The ability of these extracts to effectively neutralize hydroxyl radicals, which are among the most reactive and damaging free radicals, suggests their potential to prevent oxidative damage at the cellular level (Table 4).

Table 4
Hydroxyl radical percentage inhibition (%) of DES leaves extract of *C. roseus* at various concentrations

Leaves Extract	Concentrations (mg ml ⁻¹)					TUKEY TEST MEAN	IC ₅₀
	0.02	0.04	0.06	0.08	0.1		
ChCl:Citric Acid	35.8 ± 0.8	48.1 ± 0.5	56.1 ± 0.6	61.5 ± 0.7	66.9 ± 0.6	53.71 ^b	0.05
ChCl:Urea	8.83 ± 0.4	11.9 ± 0.4	13.8 ± 0.3	16.6 ± 0.4	19.2 ± 1.7	14.08 ^d	-
ChCl:Glycerol	21.2 ± 0.5	25.7 ± 0.6	29.8 ± 0.3	36.1 ± 0.4	46.2 ± 0.8	31.84 ^c	0.12
ChCl:Glucose	15.4 ± 0.7	18.7 ± 0.4	22.8 ± 0.3	28.3 ± 0.5	32.9 ± 0.5	23.65 ^c	-
Methanol	37.3 ± 1.3	49.8 ± 0.6	56.9 ± 0.3	62.7 ± 0.7	68.2 ± 1.1	54.98 ^b	0.05
Ascorbic acid (Standard)	46.3 ± 0.9	61.1 ± 0.4	68.0 ± 0.7	72.1 ± 0.3	75.9 ± 0.9	65.03 ^a	0.03
IC ₅₀ signifies concentrations (mg ml ⁻¹) for a 50% inhibition.							
Experiments were replicated three times and represented as mean ± standard deviation.							
The mean values having the same superscript are non-significant (p > 0.05) as per the Tukey multiple range test.							

Comparative Analysis of Different Solvent Extracts

The choice of solvent plays a critical role in determining the extraction efficiency and antioxidant properties of *Catharanthus roseus* extracts. In this study, methanol and a deep eutectic solvent (ChCl: citric acid) emerged as the most effective, producing extracts with the highest antioxidant activities. This high efficiency is largely due to the polarity of these solvents, which enhances the extraction of a wide range of bioactive compounds, especially phenolics and flavonoids, known for their potent antioxidant properties.

In contrast, extracts obtained using citric acid alone exhibited moderate antioxidant activities, highlighting the importance of selecting an optimal solvent to maximize the extraction of target phytochemicals. Among the solvents tested, deep eutectic solvents (DES), particularly ChCl: citric acid, proved to be a promising alternative to traditional solvents, offering high extraction efficiency and environmental benefits. Further confirmation of the presence of key bioactive compounds, such as phenols, flavonoids, and alkaloids, was achieved through GC-MS analysis (Table 5). The study

also explored the electrochemical behavior of these compounds, particularly their ability to reduce DPPH, which is influenced by their redox potential.¹⁴ The lower redox potential of the DPPH°/DPPH couple compared to Fe(III)/Fe(II) suggests that flavonoids should theoretically be capable of reducing DPPH. However, steric hindrance might affect this process, as the DPPH molecule's odd electron is located on a nitrogen atom shielded by phenyl and picrazyl groups.¹³ Literature reports indicate that normal green shoots of *C. roseus* exhibit stronger antioxidant activities, with IC₅₀ values of 1.57 mg/mL for DPPH and 1.44 mg/mL for ABTS assays, aligning with the current findings. Conversely, the weakest scavenging abilities were observed in callus tissues, with IC₅₀ values exceeding 3 mg/mL for DPPH and 1.85 mg/mL for ABTS. Previous studies, including those by Bhutkar and Bhise,⁹ highlighted the antioxidant activity of *C. alba* and *C. roseus* root extracts. Furthermore, Tiong identified Vindolicine, a compound in *C. roseus*, as possessing the highest antioxidant potential in the DPPH assay, underscoring the importance of specific bioactive compounds in the plant's antioxidant activity.^{16,4, 41, 42, 43}

Table 5
The results of GC-MS of Methanol extract of leaves of *Vinca 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
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
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
31	2-Methylhexacosane	C ₂₇ H ₅₆	62.409	0.17	380
32	Isoindolinine	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
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-Epoxynaphth[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)					

Mechanistic Insights and Therapeutic Potential

The strong antioxidant activities observed in methanol and ChCl: citric acid extracts of *C. roseus* can be attributed to their high phenolic and flavonoid content. These compounds act through multiple mechanisms to exert their antioxidant effects. They can directly scavenge free radicals, chelate metal ions, and upregulate endogenous antioxidant defenses.^{25–31} The results of this study suggest that *C. roseus* extracts, particularly those obtained with methanol and ChCl: citric acid, hold significant potential as natural antioxidants. They could be developed into therapeutic agents for preventing and managing oxidative stress-related conditions, such as cardiovascular diseases, cancer, and neurodegenerative

disorders.¹⁴ The use of DES for extraction enhances the yield of bioactive compounds and aligns with the principles of green chemistry, promoting sustainable and environmentally friendly extraction processes.

3. Conclusion

The study reveals a compelling presence of bioactive phytochemicals in *Catharanthus roseus*, showcasing robust antioxidant properties. Notably, methanol and ChCl: citric acid extracts stand out for their superior antioxidant activities, highlighting their promise for formulating natural antioxidant therapies. This research is groundbreaking as it identifies potent sources of natural antioxidants and evaluates the efficacy of various deep eutectic solvents (DES) compared to traditional organic solvents. This study's novelty lies in its comprehensive analysis of *Catharanthus roseus* using a range of DES solvents, which have not been extensively explored for this purpose before. This approach addresses a significant gap in the field by offering a novel method for maximizing the extraction of valuable phytochemicals. The need for this research is underscored by the growing demand for natural, plant-based antioxidants as alternatives to synthetic compounds, which are often associated with adverse effects. By highlighting the superior antioxidant potential of specific extracts, this study provides a promising avenue for developing safer and more effective natural therapies.

Declarations

Acknowledgments

The facilities provided by the Department of Chemistry, Punjab Agricultural University, Ludhiana, Punjab, India, supported this research work.

Funding source

There are no funders to report for this submission

Availability of data and materials

We have carried out the research work and assure you that it can be provided whenever required.

Author's Contribution

Parul Sharma: Worked on the project, Drafting, reviewing, editing, and finally approving 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.

Avjot Kaur: Worked on the project and gave final approval of 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.

Declaration of Competing Interest

No potential conflict of interest was reported by the authors.

Clinical Trial: Not applicable

Consent to Participate: Not applicable

Consent to Publish declarations: Not applicable

Ethics declaration: not applicable

References

1. Abbott AP, Capper G, Davies DL, Rasheed RK, Tambyrajah V. Novel solvent properties of choline chloride/urea mixtures. *Chem Commun* 2003;9:70–71.
2. Abbott AP, Capper G, Gray S. Ionic liquid analogues formed from hydrated metal salts. *ChemPhysChem* 2006;7:803–806.
3. Abdel-Raouf N, Al-Enazi NM, Ibraheem IBM. Antioxidant and antimicrobial activity of *Catharanthus roseus*-derived deep eutectic solvents. *Arab J Chem* 2017;10:S3029-S3040.
4. Agarwal S, Chettri N, Bisoyi S. Evaluation of in-vitro anthelmintic activity of *Catharanthus roseus* extract. *Int J Pharm Sci Drug Res* 2011;3(3):211–213.
5. Ahmad T, Irfan M, Bhattacharjee S. Green synthesis of nanoparticles using *Catharanthus roseus* extract. *Procedia Eng* 2016;148:1396–1401.

6. Ahmed S, Khan M, Ullah S, Raza MM. Antioxidant potential of plant-derived deep eutectic solvents. *J Photochem Photobiol B* 2016;161:141–148.
7. Balaabirami S, Patharajan S. *In vitro*, antimicrobial, and antifungal activity of *Catharanthus roseus* leaves extract against important pathogenic organisms. *Int J Pharm Pharm Sci* 2012;4(3):487–490.
8. Barkat A, Mujeeb M. The comparative study of *Catharanthus roseus* extracts and extract-loaded chitosan nanoparticles in alloxan-induced diabetic rats. *Int J Biol Res* 2013;4(12):670–678.
9. Barthe L, Ribet JP, Pélioussou M, Degude MJ, Fahy J, Duflos A. Optimization of the separation of *Vinca* alkaloids by nonaqueous capillary electrophoresis. *J Chromatogr A* 2002;968:241–250.
10. Bennouna J, Delord JP, Campone M. Vinflunine: A new microtubule inhibitor agent. *Clin Cancer Res* 2008;14(5):1625–1632.
11. Bhutkar MA, Bhise SB. *In vitro* evaluation of antioxidant properties of *Catharanthus roseus*. *Int J PharmTech Res* 2011;3:1551–1558.
12. Dai Y, Rozema E, Verpoorte R. Application of natural deep eutectic solvents to the extraction of anthocyanins from *Catharanthus roseus* with high extractability and stability replacing conventional organic solvents. *J Chromatogr A* 2016;1434:50–56.
13. Demir A, Fesliyan S, Altunay N. Deep eutectic solvent-based sonication-assisted dispersive liquid-liquid microextraction using Box-Behnken optimization for the determination of patent blue V in food and drug samples. *J Food Compos Anal* 2024;135:106634. <https://doi.org/10.1016/j.jfca.2024.106634>.
14. Gaur A, Ganeshan M, Shah R. Microbiological, pharmacological and phytochemical efficacy of *Catharanthus roseus* (L.) G. Don. *Int J Pure Appl Biosci* 2016;4(4):102–113.
15. Javad S, Sarwar S, Jabeen K. Enhanced extraction of an anticancer drug, vinblastine, from *Catharanthus roseus*. *Pure Appl Biol* 2016;5(3):608–614.
16. Jeewantha HA, Ivanovich SA, Mihailovich MP. Bioactivity-guided isolation of antidiabetic compounds from *Catharanthus roseus*. *Int J Pharm Pharm Sci* 2017;9:1–7.
17. Kaur A, Kaur R, Bhardwaj U, Sharma P. Optimisation and utilization of deep eutectic solvents for the green extraction of anticancer alkaloids from *Catharanthus roseus*. *Nat Prod Res* 2025;1–9. <https://doi.org/10.1080/14786419.2025.2476199>.
18. Kaur R, Kaur R, Singh R, Bhardwaj U, Sharma P. Chemical composition and antifungal potential of *Vinca rosea* leaf essential oil and extracts from Northern India. *J Biol Active Plants Nat* 2024;12:551–562. <https://doi.org/10.1080/22311866.2024.2434569>.
19. Khatami M, Amini A, Tavakkoli Yarak M, Pourseyedi S, Hamblin MR. Green synthesis of metallic nanoparticles using *Catharanthus roseus*. *J Cleaner Prod* 2019;208:1171–1180.
20. Kiruba Daniel SCG, Elangovan A, Jayaprakash R, Das J. Green synthesis of nanoparticles using plant extracts. *J Nanopart Res* 2012;15:1–9.
21. Kora AJ, Rastogi L. Biogenic synthesis of silver nanoparticles using *Catharanthus roseus*. *Ind Crops Prod* 2016;81:1–8. Abbott AP, Capper G and Davies DL. 2003. Novel solvent properties of choline chloride/urea mixtures. *Chem Commun.* 9(1):70–71. <https://doi.org/10.1039/B210714G>.
22. Li Y, Pan Z, Wang B, Yu W, Song S, Feng H, Zhao W, Zhang J. Ultrasound-assisted extraction of bioactive alkaloids from *Phellodendri amurensis* cortex using deep eutectic solvent aqueous solutions. *New J Chem* 2020;44:9172–9180.
23. Milardovic S, Ivekovic D, Grabaric BS. Electrochemical properties of bioactive compounds extracted from *Catharanthus roseus*. *Bioelectrochemistry* 2006;68:175–181.
24. Patil PJ, Ghosh JS. Antimicrobial activity of *Catharanthus roseus*: A detailed study. *Br J Pharmacol Toxicol* 2010;1(1):40–44.
25. Rajput MS, Nair V, Chauhan A. Evaluation of antidiarrheal activity of aerial parts of *Vinca major* in experimental animals. *Middle-East J Sci Res* 2011;7(5):784–788.
26. Rawat S, Gupta A. Development and study of wound healing activity of an Ayurvedic formulation. *Asian J Res Pharm Sci* 2011;1(1):26–28.
27. Salamat Q, Soylak M. Novel reusable and switchable deep eutectic solvent for extraction and determination of curcumin in water and food samples. *Talanta* 2023;125401. <https://doi.org/10.1016/j.talanta.2023.125401>.
28. Shang X, Tan JN, Du Y, Liu X, Zhang Z. Green extraction of flavonoids from *Cyclocarya paliurus* leaves with deep eutectic solvents. *Molecules* 2018;23:1046–1058.
29. Shang X, Tan JN, Du Y. Environmentally-friendly extraction of flavonoids from *Cyclocarya paliurus* (Batal.) Iljinskaja leaves with deep eutectic solvents and evaluation of their antioxidant activities. *Molecules* 2018;23(2):1046.
30. Sharma P, Kaur R, Bhardwaj U, Kaur J. Chemical composition and antifungal potential of *Vinca rosea* leaf essential oil. *Cogent Food Agric* 2024;10:2382317. <https://doi.org/10.1080/23311932.2024.2382317>.
31. 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* 2024;12:131–156. <https://doi.org/10.22271/plants.2024.v12.i3b.1675>.
32. Sharma P, Singla N, Kaur R. A review on phytochemical constituents and pharmacological properties of *Catharanthus roseus* (L.) G. Don. *J Med Plants Stud* 2024;12(3):131–156. <https://doi.org/10.22271/plants.2024.v12.i3b.1675>.

33. Smink D, Juan A, Schuur B, Kersten SRA. Understanding the role of choline chloride in deep eutectic solvents used for biomass delignification. *Ind Eng Chem Res* 2019;58:16348–16357.
34. Tan T, Zhang M, Wan Y, Qiu H. Utilization of deep eutectic solvents as novel mobile phase additives for improving separation of bioactive quaternary alkaloids. *Talanta* 2016;149:85–90.
35. Tang B, Park HE, Row KH. Simultaneous extraction of flavonoids from *Chamaecyparis obtusa* using deep eutectic solvents as additives of conventional extractions solvents. *J Chromatogr Sci* 2014;53(6):836–840.
36. Tiong SH, Looi CY, Hazni H, Arya A, Paydar M, Wong WF, Cheah SC. Antidiabetic and antioxidant properties of alkaloids from *Catharanthus roseus*. *Molecules* 2013;18:9770–9784.
37. Vega JT, Gómez-Alons S, Pérez-Navarro J. Green extraction of alkaloids and polyphenols from *Peumus boldus* leaves with natural deep eutectic solvents and profiling by HPLC-PDA-IT-MS/MS and HPLC-QTOF-MS/MS. *Molecules* 2020;25(2):242.
38. Wang M, Wang JQ, Zhou YY, Zhang MY, Xia Q, Bi WT, Chen DDY. Environmentally friendly solvent systems for alkaloid extraction. *ACS Sustain Chem Eng* 2017;5:6297–6304.
39. Xu M, Ran L, Chen N, Fan X, Ren D, Yi L. Antioxidant activity and extraction efficiency of deep eutectic solvents. *Food Chem* 2019;297:124970.
40. Yao XH, Zhang DY, Duan MH, Cui Q, Xu WJ, Luo M, Li CY, Zu YG, Fu YJ. Deep eutectic solvent-based extraction of bioactive compounds. *Sep Purif Technol* 2015;149:116–124.
41. Zainal-Abidin MH, Hayyan M, Hayyan A, Jayakumar NS. Deep eutectic solvent for bioactive compound extraction. *Anal Chim Acta* 2017;979:1–9.
42. Zhou PF, Wang XP, Liu PZ, Huang J, Wang C, Pan M, Kuang ZS. Industrial applications of deep eutectic solvents in phytochemical extraction. *Ind Crops Prod* 2018;120:147–156.
43. Zhuang B, Dou LL, Li P, Liu EH. Advances in quality control of traditional Chinese medicines by high-performance liquid chromatography. *J Pharm Biomed Anal* 2016;134:214–229.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupportingInformation.docx](#)