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Evaluation the Antibacterial, Antioxidant Activity and Phytochemical Screening Profile of Catharanthus roseus

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ABSTRACT: The increasing emergence of antimicrobial-resistant bacteria has encouraged the exploration of medicinal plants as alternative sources of bioactive compounds with therapeutic potential. A study was conducted to assess the antibacterial and antioxidant activity of Catharanthus roseus and analyze its phytochemical profile. The plant extract exhibited significant antioxidant activity with an IC_{50} value of 119.3 ppm and was able to inhibit the growth of both Gram-positive and Gram-negative bacteria that causes UTIs. Alkaloids were identified as vincristine, vinblastine, catharanthine and the major phenolic compounds were identified as quercetin, rutin, ferulic acid, p-coumaric acid, vitexin, kaempferol and caffeic acid by HPLC. These results suggest that C. roseus could be used as a natural source of bioactive compounds that are potentially used in pharmaceuticals.

I. INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide and represent a major public health concern affecting individuals of all age groups. These infections account for millions of healthcare visits annually and are associated with considerable morbidity, healthcare costs, and reduced quality of life. Gram-negative bacteria, particularly Escherichia coli, are the predominant causative agents of UTIs, followed by *K. pneumoniae*, *P. aeruginosa*, and several Gram-positive species. Although antibiotics remain the primary treatment for UTIs, the increasing emergence of antimicrobial-resistant pathogens has become a major challenge to effective disease management and has encouraged the search for alternative therapeutic agents [1,2]. Medicinal plants have long been recognized as valuable sources of bioactive compounds with diverse pharmacological properties. Plant-derived secondary metabolites, including phenolic compounds, flavonoids, alkaloids, terpenoids, and glycosides, exhibit antioxidant, antimicrobial, anti-inflammatory, and anticancer activities. Consequently, medicinal plants have attracted increasing scientific interest as potential sources for the development of novel pharmaceutical agents with improved safety and therapeutic efficacy [3,4]. Oxidative stress plays an important role in the development and progression of many infectious and chronic diseases through the excessive production of reactive oxygen species. Natural antioxidants derived from medicinal plants can neutralize free radicals and reduce oxidative damage to biological molecules. Phenolic compounds and flavonoids are considered among the principal phytochemicals responsible for the antioxidant capacity of medicinal plants owing to their electron-donating and free radical scavenging abilities [5,6]. *C. roseus* (L.) G. Don, a member of the family Apocynaceae, is a medicinal plant widely distributed in tropical and subtropical regions. Besides its well-known ornamental value, the plant is an important natural source of pharmacologically active constituents, particularly the indole alkaloids vincristine, vinblastine, and catharanthine, in addition to phenolic compounds and flavonoids. Previous studies have demonstrated that *C. roseus* possesses antioxidant, antimicrobial, anti-inflammatory, and anticancer activities, highlighting its considerable pharmaceutical potential [7,8]. Despite the extensive pharmacological importance of *C. roseus*, information concerning the antioxidant activity, antibacterial efficacy, and phytochemical profile of its different extracts remains limited. Therefore, the present study aimed to evaluate the antibacterial and antioxidant activities of *C. roseus* extracts and characterize their bioactive constituents using phytochemical analysis and high-performance liquid chromatography (HPLC).

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II. MATERIALS AND METHODS

Plant Material: Aerial parts (leaves and stems) of *C. roseus* were obtained from Al-Zohoor Nursery in Wasit Province of Iraq. The plant material was washed, dried in the oven at 37ºC, ground to a fine powder and preserved for extraction.

Plant Constituents Extraction: Terpenoid and alkaloid extracts were prepared as per Harborne (1984) while phenolic compounds were extracted as per Ribéreau-Gayon (1972). Concentration of the extracts was carried out by using a rotary evaporator and stored at 4°C for further analysis.

Quantification of Bioactive Compounds: Total phenolics, flavonoids, alkaloids, tannins, glycosides and saponins were quantified as described by Al-Salami (1998), Al-Bid (1985), Baba and Malik (2015), Ghorai et al. (2012) and Tofighi and Ghazi Saeedi (2016)

Antioxidant Activity: The antioxidant activity of the crude extract was evaluated using the DPPH free radical scavenging assay according to Yen and Duh (1994) and Von Gadow et al. (1997).

Antibacterial Activity: The antibacterial activity of the extracts was determined using the agar well diffusion method according to Balouiri et al. (2016). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined according to Wiegand et al. (2008), and the results were interpreted following CLSI (2025).

HPLC Analysis: The identification and quantification of alkaloid and phenolic compounds were carried out using HPLC according to Ngamsuk et al. (2020) and Krstonošić et al. (2019).

Statistical Analysis: All experiments were performed in triplicate. Data were analyzed using SPSS version 26. Mean differences were compared using one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test at $P \leq 0.05$ according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Yield of Plant Extracts: The extraction yields of terpenoid, phenolic, and alkaloid extracts obtained from the aerial parts of *C. roseus* are presented in Table 1. The alkaloid extract showed the highest extraction yield (19.8%), followed by the phenolic extract (14.2%), whereas the terpenoid extract exhibited the lowest yield (6.5%). The variation in extraction yield may be attributed to differences in the distribution of secondary metabolites and extraction efficiency. Similar observations have been reported by Verma et al. (2007), Rajashekara et al. (2022), and Islam et al. (2022).

Table 1. Yield (%) of different extracts obtained from *C. roseus*.

Extract type	Yield (%)
Terpenes	6.5
Phenols	14.2
Alkaloids	19.8

Quantification of Bioactive Compounds: The quantitative analysis of bioactive compounds in *C. roseus* is presented in Table 2. Total phenolic content (TPC) was the predominant constituent (126.8 mg/g), followed by total flavonoid content (74.3 mg/g). Lower concentrations were recorded for alkaloids (3.84 mg/g), tannins (2.11 mg/g), glycosides (1.26 mg/g), and saponins (0.42 mg/g). The predominance of phenolic compounds may explain the biological activities of the plant. Similar findings have been reported for *C. roseus* by Nisar et al. (2017) and Tolambiya et al. (2023).

Table 2. Quantification of bioactive compounds in ***C. roseus***.

Bioactive compound	Content (mg/g)
Total phenolics (TPC)	126.8
Total flavonoids (TFC)	74.3
Alkaloids	3.84

Glycosides	1.26
Tannins	2.11
Saponins	0.42

Antioxidant Activity: The antioxidant activity of the crude extract of *C. roseus* increased in a concentration-dependent manner, reaching the highest DPPH radical scavenging activity at 500 ppm. The calculated IC_{50} value was 116.9 ppm (Table 3), indicating strong antioxidant potential. The antioxidant activity of the crude extract may be attributed to the combined effect of phenolic compounds, flavonoids, alkaloids, and other phytochemicals present in the plant. Similar findings have been reported by Lobo et al. (2010) and Salehi et al. (2019).

Table 3. DPPH radical scavenging activity of ***C. roseus*** crude extract.

Concentration (ppm)	**Inhibition (%)**
30	21.58
60	31.67
120	52.84
250	64.31
500	80.65
IC_{50} (ppm)	**116.9**

Antibacterial Activity :The antibacterial activities of the terpenoid, phenolic, and alkaloid extracts of *C. roseus* against the tested bacterial isolates are presented in Table 4. The phenolic extract exhibited the highest antibacterial activity at all tested concentrations, followed by the alkaloid extract, whereas the terpenoid extract showed the lowest activity. At 75 mg/ml, the phenolic extract produced inhibition zones of **24.6 ± 0.8**, **20.8 ± 0.7**, **19.6 ± 0.6**, and **18.7 ± 0.6 mm** against *S. aureus*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae*, respectively. Significant differences were observed among the extract types at all tested concentrations ($P = 0.001$). The superior antibacterial activity of the phenolic extract may be attributed to its high phenolic content, which has been reported to damage bacterial cell membranes and inhibit essential metabolic processes. Similar findings were reported by Shoker et al. (2021), Solaq and Shoker (2026), and Raheema and Hassan (2016).

Table 4. Antibacterial activity of ***C. roseus*** extracts against the tested bacterial isolates (mean ± SD).

Bacteria	**Extract type**	**25 mg/ml**	**50 mg/ml**	**75 mg/ml**
S. aureus	Terpene	0.0 ± 0.0 C	0.0 ± 0.0 C	8.6 ± 0.4 C
	Alkaloid	12.1 ± 0.5 B	16.3 ± 0.6 B	20.4 ± 0.7 B
	Phenol	14.2 ± 0.6 A	19.5 ± 0.6 A	24.6 ± 0.8 A
E. coli	Terpene	0.0 ± 0.0 C	0.0 ± 0.0 C	7.1 ± 0.3 C
	Alkaloid	10.5 ± 0.5 B	14.2 ± 0.6 B	17.6 ± 0.6 B
	Phenol	12.7 ± 0.5 A	16.7 ± 0.6 A	20.8 ± 0.7 A
P. aeruginosa	Terpene	0.0 ± 0.0 C	0.0 ± 0.0 C	6.5 ± 0.3 C
	Alkaloid	9.8 ± 0.4 B	13.5 ± 0.5 B	16.9 ± 0.5 B
	Phenol	11.5 ± 0.4 A	15.9 ± 0.6 A	19.6 ± 0.6 A
K. pneumoniae	Terpene	0.0 ± 0.0 C	0.0 ± 0.0 C	6.5 ± 0.3 C
	Alkaloid	9.2 ± 0.4 B	12.8 ± 0.5 B	16.1 ± 0.5 B

	Phenol	10.9 ± 0.4 A	15.1 ± 0.5 A	18.7 ± 0.6 A
LSD		**1.07**	**1.34**	**1.44**
P-value		**0.0019**	**0.0006**	**<0.0001**

Different letters within the same column indicate significant differences at P ≤ 0.05

Comparison with Standard Antibiotics: The antibacterial activity of phenolic extract from *C. roseus* was compared with standard antibiotics against the tested bacterial isolates (Table 5). The phenolic extract had significant antibacterial activities against all the tested bacteria despite the larger inhibition zone exhibited by the standard antibiotics. The zone of inhibition for the highest activity was obtained with *S. aureus* (24.6 ± 0.8 mm) and the lowest zone of inhibition was received with *K. pneumoniae* (18.7 ± 0.6 mm). The effectivity of the antibiotics is anticipated due to the presence of the purified antimicrobial agents in the antibiotics, while the plant extract consists of a blend of antimicrobial compounds in a crude form. However, the high antibacterial activity of the phenolic extract indicates the possibility of using this extract as a natural source of antibacterial agents. Similar antibacterial activity of plant phenolic extracts has been reported by Shoker et al. (2021) and Solaq and Shoker (2026).

Table 5. Comparison of the antibacterial activity of the phenolic extract of ***C. roseus*** with standard antibiotics.

Treatment	***S. aureus***	***E. coli***	***P. aeruginosa***	***K. pneumoniae***
Ciprofloxacin (5 µg/disc)	28.4 ± 0.9 B	26.7 ± 0.8 B	25.5 ± 0.7 B	25.1 ± 0.7 B
Linezolid (30 µg/disc)	30.2 ± 0.8 A	—	—	—
Amikacin (30 µg/disc)	—	27.9 ± 0.8 A	26.8 ± 0.7 A	26.1 ± 0.7 A
C. roseus Phenolic Extract (75 mg/ml)	24.6 ± 0.8 C	20.8 ± 0.7 C	19.6 ± 0.6 C	18.7 ± 0.6 C
LSD	**1.52**	**1.41**	**1.33**	**1.29**
P-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Different antibacterial activities were observed between the phenolic extract and the standard antibiotics against the tested bacterial isolates.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC): The phenolic extract of *C. roseus* exhibited a minimum inhibitory concentration (MIC) of 6.25 mg/ml and a minimum bactericidal concentration (MBC) of 12.5 mg/ml against *S. aureus* (Table 6). The MBC/MIC ratio was 2, showing that the phenolic extract was bactericidal against the tested isolate. Low MIC and MBC values indicate the potent antibacterial activity of the phenolic extract, likely due to its rich phenolic composition that helps disrupt bacterial cell membranes and inhibit key metabolic pathways. Similar antibacterial effects of phenolic and alkaloid plant extracts have been reported by Raheema and Hassan (2016), while Wiegand et al. (2008) and Balouiri et al. (2016) described the methodological basis for MIC and antibacterial susceptibility testing.

Table 6. MIC and MBC of the phenolic extract of ***C. roseus*** against ***S. aureus***.

Plant extract	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC ratio
C. roseus (Phenolic extract)	6.25	12.5	2

HPLC Analysis: The HPLC profiles of the alkaloid reference standards are shown in Figures 1–3. The three alkaloid compounds were identified in the *C. roseus* extract by comparing their retention time with the corresponding reference standards. The HPLC chromatogram of the alkaloid extract is shown in Figure 4 and the amounts of the identified compounds are summarized in Table 7. The predominance of vinblastine and vincristine in the composition indicates the characteristic alkaloid composition of *C. roseus* that is known to yield pharmacologically active indole alkaloids. The same observation has been reported by Verma et al. (2007), Rajashekara et al. (2022), and Solaq and Shoker (2026).

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Table 7. HPLC analysis of alkaloid compounds identified in ***C. roseus***.

Compound	Concentration (ppm)
Vincristine	365.08
Vinblastine	412.56
Catharanthine	189.87
Total	**967.51**

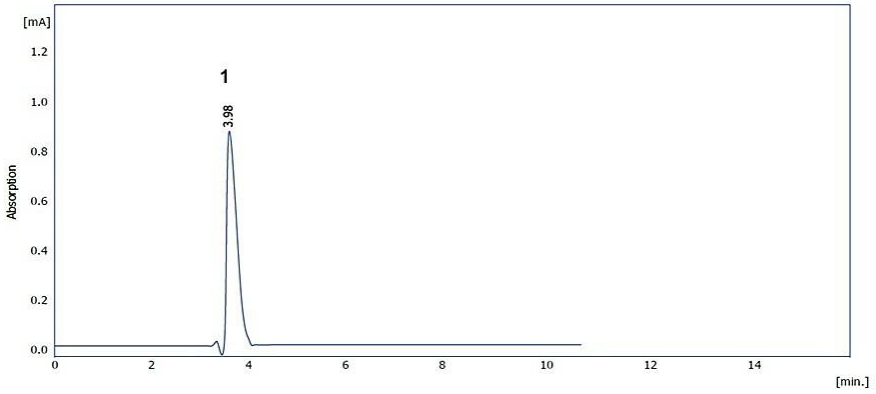

Figure 1. HPLC profile of alkaloid standard of C. roseus plant (1) Vincristine.

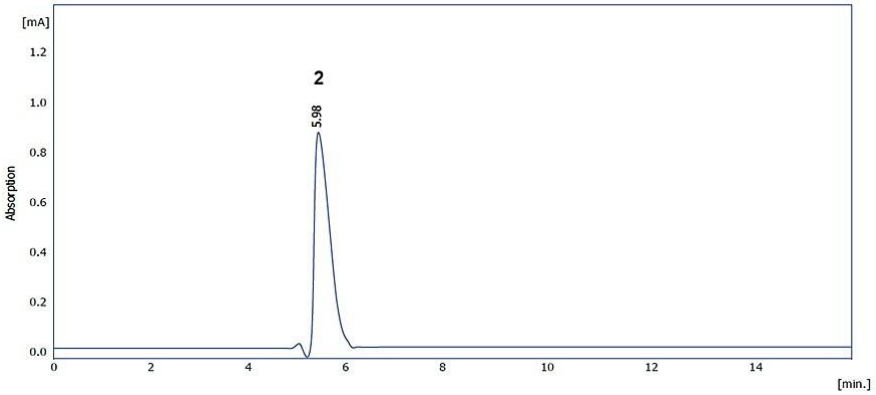

Figure 2. HPLC profile of alkaloid standard of C. roseus plant (2) Vinblastine.

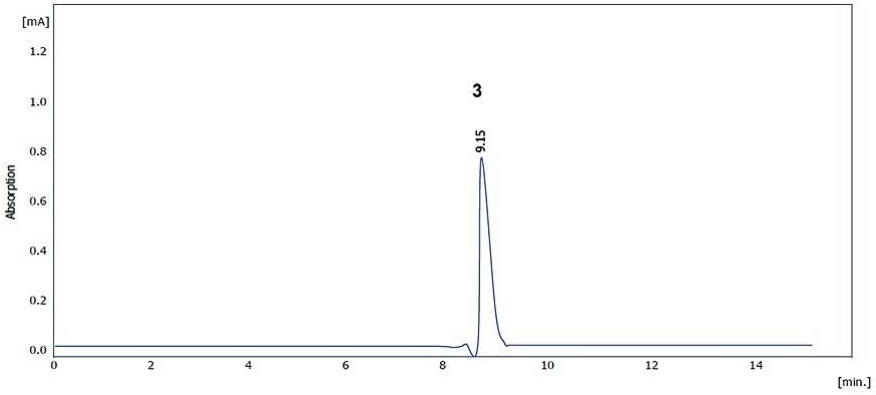

Figure 3. HPLC profile of alkaloid standard of C. roseus plant (3) Catharanthine.

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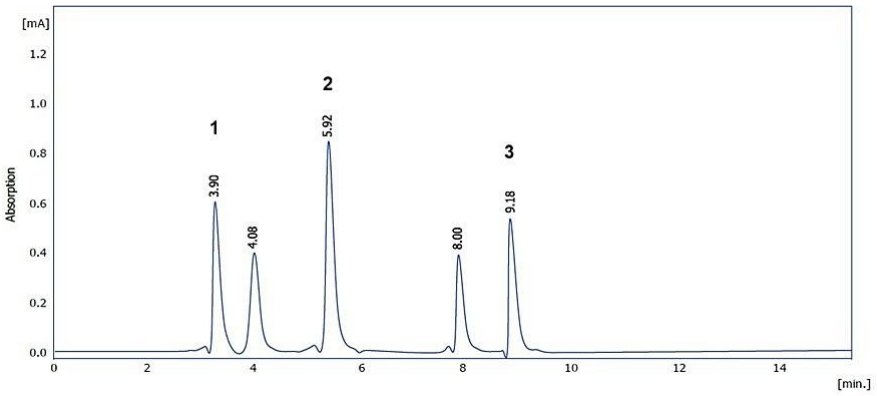

Figure 4. HPLC profile of C. roseus alkaloid extract (1) Vincristine, (2) Vinblastine, (3) Catharanthine.

The HPLC profiles of the phenolic reference standards are shown in Figures 5–7. Phenolic compounds in the *C. roseus* extract were identified by comparing their retention times with those of the corresponding reference standards. The HPLC chromatograms of the phenolic extract are shown in Figures 8–11 and the concentrations of the identified compounds are summarized in Table 8. The major phenolic compounds found were quercetin and rutin, while ferulic acid was also present and showed the characteristic phenolic profile of *C. roseus*. These phenolic constituents are known for their antioxidant and antimicrobial properties and may contribute to the biological activities observed in the present study. Similar findings have been reported by Shoker et al. (2021), Rajashekara et al. (2022), and Aisyah et al. (2023).

Table 8. HPLC analysis of phenolic compounds identified in ***C. roseus***.

Phenolic compound	Concentration (ppm)
Caffeic acid	52.6
Ferulic acid	90.7
Kaempferol	58.7
p-Coumaric acid	86.7
Quercetin	129.1
Rutin	112.5
Vitexin	79.8
Total	610.1

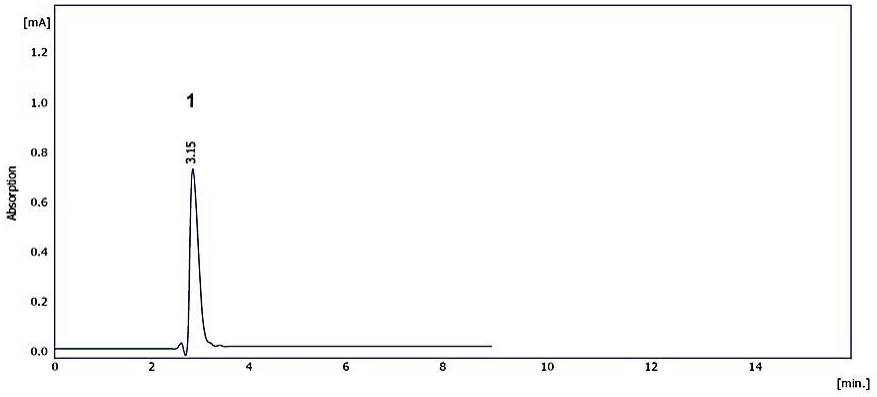

Figure 5. HPLC profile of phenolic standard of *C. roseus* plant (1) Vitexin.

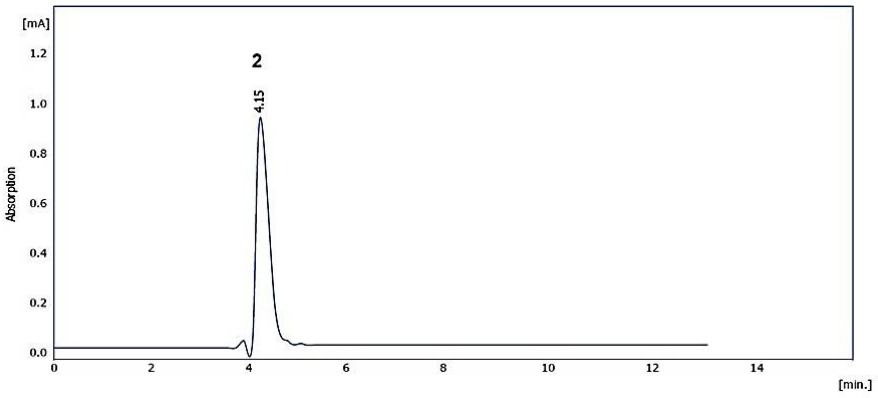

Figure 6. HPLC profile of phenolic standard of *C. roseus* plant (2) Rutin.

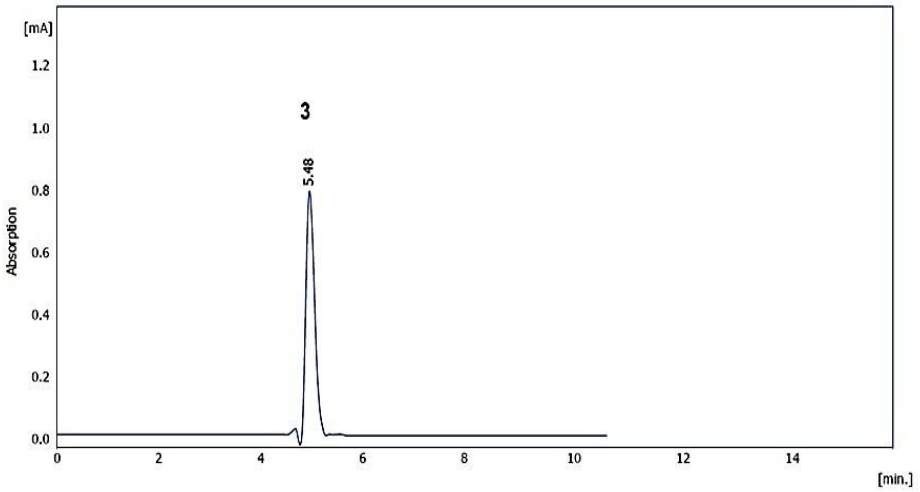

Figure 7. HPLC profile of phenolic standard of *C. roseus* plant (3) Kaempferol.

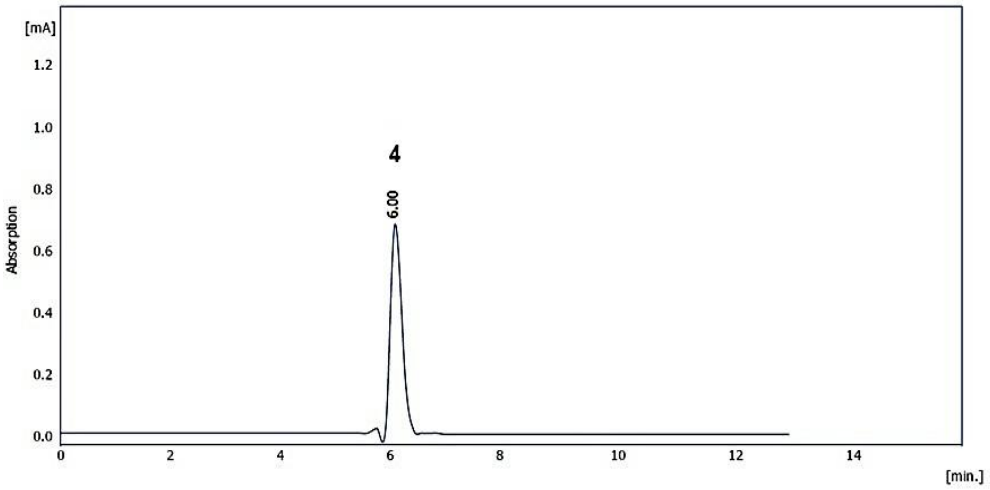

Figure 8. HPLC profile of phenolic standard of *C. roseus* plant (4) Ferulic acid.

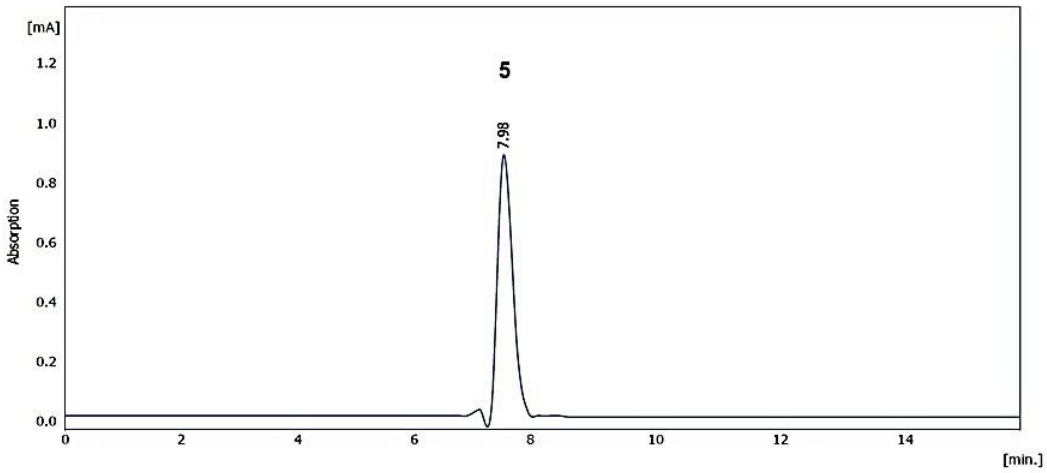

Figure 9. HPLC profile of phenolic standard of *C. roseus* plant (5) Quercetin.

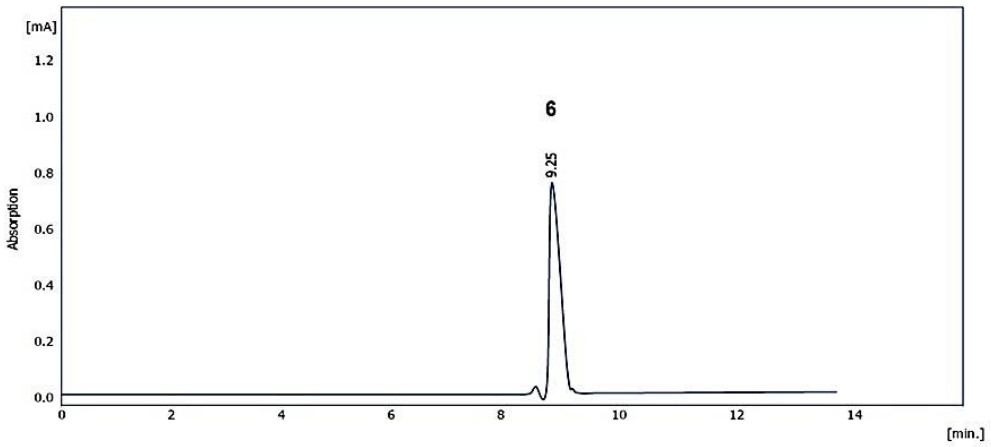

Figure 10. HPLC profile of phenolic standard of *C. roseus* plant (6) Caffeic acid.

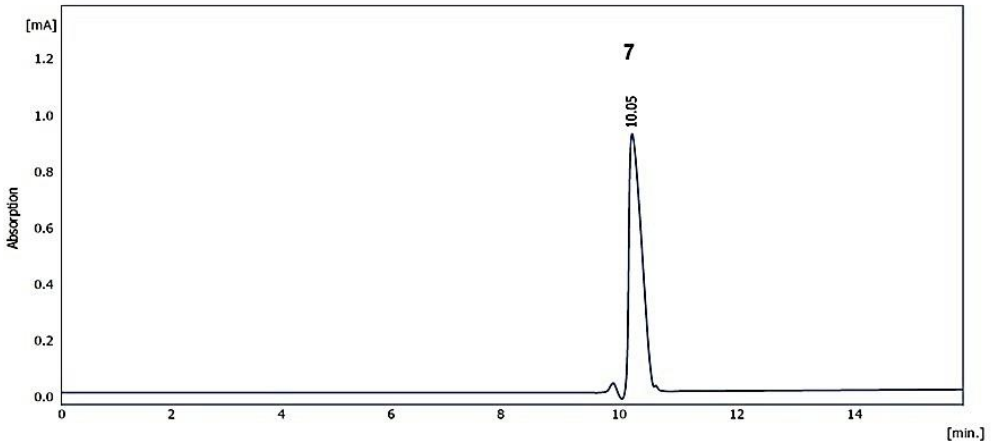

Figure 11. HPLC profile of phenolic standard of *C. roseus* plant (7) *p*-Coumaric acid

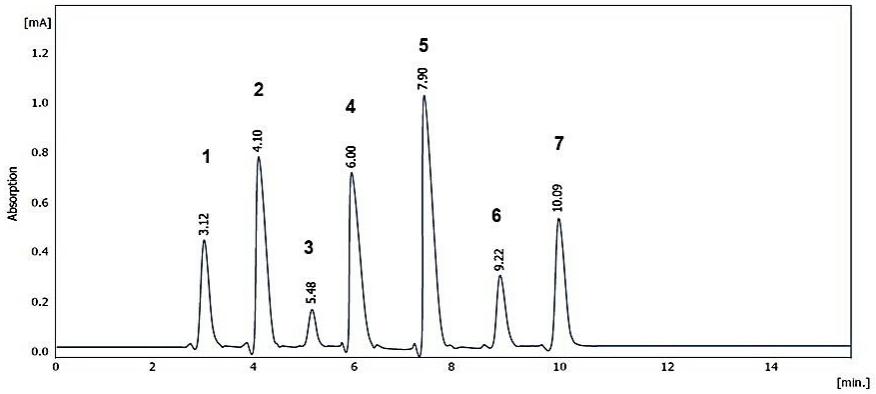

Figure 11. HPLC profile of *C.s roseus* alkaloid extract (1) Vitexin, (2) Rutin, (3) Kaempferol, (4) Ferulic acid, (5) Quercetin, (6) Caffeic acid, (7) *p*-Coumaric acid.

III. CONCLUSIONS

The present study demonstrated that *C. roseus* is a rich source of bioactive compounds with promising antioxidant and antibacterial activities. The phenolic extract exhibited the highest antibacterial activity against the tested bacterial isolates, while the crude extract showed strong antioxidant activity in the DPPH assay. HPLC analysis confirmed the presence of major alkaloids, including vincristine, vinblastine, and catharanthine, as well as phenolic compounds such as quercetin, rutin, ferulic acid, p-coumaric acid, caffeic acid, kaempferol, and vitexin, which may collectively contribute to the observed biological activities. These findings suggest that *C. roseus* has considerable potential as a natural source of bioactive compounds for future pharmaceutical applications. Further *in vivo* studies and toxicity evaluations are recommended to confirm its therapeutic efficacy and safety.

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