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Phytochemical profiling, HPLC analysis, and antimicrobial potential of *Curio radicans* (L. f.) P.V. Heath

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Medicinal plants are dynamic reservoirs of bioactive molecules, offering immense potential for novel therapeutic discoveries. This study explores the phytochemical and antimicrobial potential of *Curio radicans* through detailed qualitative, quantitative, and HPLC analysis. Phytochemical screening revealed the ethanolic extract to be particularly rich in alkaloids, flavonoids, terpenoids, saponins, tannins, lipids, and phenols, whereas the ethyl acetate extract showed higher concentrations of phenolics and flavonoids. Quantitative estimations confirmed that the ethanolic extract possessed significantly higher levels of alkaloids (7.76 mg/g), flavonoids (7.60 mg/g), and tannins (10.32 mg/g) compared to the ethyl acetate extract (3.51, 1.33, and 2.56 mg/g, respectively). HPLC profiling further supported these findings, identifying abundant phenolic acids and flavonoids with well-documented pharmacological relevance. The ethanolic extract contained catechin, fumaric acid, hydroxybenzoic acid, caffeic acid, and salicylic acid, while the ethyl acetate extract was enriched with vanillin, protocatechuic acid, ellagic acid, caffeic acid, and p-coumaric acid. Antimicrobial assays demonstrated remarkable dose-dependent inhibition against both Gram-positive and Gram-negative bacteria, with *Escherichia coli* being highly susceptible (17.40 ± 1.15 mm). Fungal strains, particularly *Aspergillus niger*, also showed significant sensitivity (15.27 ± 0.39 mm). Collectively, these results validate the ethnomedicinal importance of *C. radicans* and underscore its potential as a natural source of bioactive compounds for pharmaceutical development.

Keywords Phytochemical profiling, HPLC analysis, Antimicrobial activity, *Curio radicans*

Antimicrobial resistance has emerged as one of the most pressing global health threats, with bacterial and fungal pathogens increasingly developing resistance to conventional antibiotics[1]. Infectious diseases remain highly prevalent in developing regions, disproportionately affecting vulnerable populations such as children and the elderly. Common bacterial pathogens—including *Campylobacter*[2]*Salmonella*, *Shigella*[3]*Shigella*[4] and *Escherichia coli*[5] continue to cause significant morbidity worldwide, while fungal pathogens such as *Aspergillus*, *Candida*, and *Cryptococcus* contribute to over 1.5 million deaths annually[6,7]. The World Health Organization has classified several of these microorganisms as high priority and critical pathogens, underscoring the urgent need to discover novel antimicrobial agents[8,9].

Medicinal plants represent a promising alternative in this search, as they are rich sources of bioactive secondary metabolites[6]. These compounds, which include terpenoids, alkaloids, flavonoids, tannins, saponins, glycosides, and phenolics, are synthesized as defense mechanisms against environmental stressors and microbial attack[10]. Beyond their ecological role, they exhibit diverse pharmacological properties, including antibacterial, antifungal, antioxidant, anti-inflammatory, and anticancer activities[11]. Modern analytical tools, particularly high-performance liquid chromatography (HPLC), gas chromatography (GC), and gas chromatography–mass spectrometry (GC-MS), have facilitated the rapid identification and characterization of such metabolites, although GC-MS often provides superior sensitivity and structural elucidation for complex mixtures[12–14].

Curio radicans (L. f.) P.V. Heath, commonly known as String of Bananas or Fish Hooks Senecio, is a trailing succulent belonging to the family Asteraceae. Native to Namibia, Lesotho, and South Africa, it also grows in western Pakistan, especially Balochistan, where it thrives in arid and semi-arid regions. Traditionally, *C. radicans* has been used for wound healing and anti-inflammatory purposes, and recent pharmacological studies report antioxidant and genoprotective activities of its extracts, including protection against H_2O_2-induced DNA damage

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in human lymphocytes. GC-MS analysis has revealed bioactive compounds such as 3,4-bis(methoxycarbonyl) furan, 4-imidazolidinone, and diisooctyl phthalate, highlighting its therapeutic relevance. Despite belonging to the well-studied Asteraceae family, *C. radicans* remains largely underexplored, presenting a unique opportunity to expand phytochemical and pharmacological knowledge of this species[15–17]the reason, *C. radicans* was selected for the current study.

This study investigates the bioactive compounds of *C. radicans* through phytochemical screening and HPLC analysis, while also evaluating its antimicrobial potential. In the context of rising antimicrobial resistance and limited scientific reports on this species, the present work addresses an important research gap and highlights the potential of *C. radicans* as a source of novel therapeutic agents.

Methodology

Plant collection

The plant *Curio radicans* was collected in March 2024 from Quetta, Balochistan, Pakistan, located at coordinates 30.183270° N and 66.996452° E. Its identification was carried out using the *Flora of Pakistan* and verified by Ghulam Jelani, a plant taxonomist. The plant was collected in accordance with the guidelines issued under the IUCN policy. A voucher specimen, labeled Muhammad Naseer Bot.2050 (QUP), was deposited in the herbarium for future reference.

Plant extraction

The collected plant material was thoroughly washed with water and air-dried under shade at room temperature to prevent the loss of heat-sensitive compounds. The dried material was then finely ground into powder. A 50-gram portion of this powder was soaked in 300 mL of ethanol and ethyl acetate solutions, which were procured from U.M. Enterprises. After maceration for 48 h, the mixtures were first filtered through muslin cloth and then through filter paper. The obtained filtrates were concentrated using a rotary vacuum evaporator (RE-100D Phoenix) manufactured by MED Lab Services. The ethyl acetate extract yielded 10.5 g, while the ethanol extract yielded 10.13 g. Both extracts of *Curio radicans* were stored in sealed bottles at 4 °C for future use[18].

Qualitative phytochemical screening

Qualitative phytochemical screening of the extracts was conducted following standard protocols with minor modifications to suit the study objectives. The presence of saponins was indicated by stable, persistent foam formation from a boiled aqueous extract, which produced an emulsion upon addition of olive oil[19]. Oils and fats were confirmed by the appearance of a translucent oily spot when the powdered sample was pressed between filter papers[20]. Gallic tannins yielded a characteristic blue coloration with ferric chloride, while a greenish-black hue signified catechol tannin[21]. Alkaloids, assessed via Mayer's test, were detected through the formation of a creamy white precipitate[22]. Cardiac glycosides (Keller–Kiliani test) were confirmed by a distinctive brown ring at the interface of glacial acetic acid/ferric chloride and concentrated sulfuric acid layers, with subsequent violet and green rings appearing below[23]. Phytosterols were identified by a brown interface ring and a progressive color shift from light green to dark green following treatment with acetic anhydride and concentrated sulfuric acid[24]. Terpenoids produced a reddish-brown interface upon reaction of the extract with chloroform and sulfuric acid[20]. Flavonoids were evidenced by an immediate red coloration upon treatment of the ethanolic extract with concentrated hydrochloric acid[20]. Phenolic compounds were indicated by blue, green, red, or purple coloration in the presence of ferric chloride[20]. Steroids were confirmed by a violet-to-blue or green transition after treatment with acetic anhydride and sulfuric acid[23]. Proteins were detected through the development of a violet color upon reaction with ninhydrin solution[25]. A small amount of extract was mixed with a few drops of α-naphthol solution, followed by the addition of concentrated sulfuric acid along the side of the test tube. The formation of a violet ring at the interface indicated the presence of carbohydrates[26]. A small quantity of powdered sample was pressed between two filter papers. The appearance of a translucent oily spot confirmed the presence of lipids[26].

Quantitative phytochemical analysis

Quantification of saponin

A 5-gram powder sample was mixed with 50 mL of 20% ethanol solution, stirred for 30 min, and heated in a water bath at 55 °C for four hours. The mixture was filtered through Whatman filter paper. For a second extraction, the remaining residue was treated with 200 mL of 20% aqueous ethanol. The filtrates from both extractions were combined and concentrated in a water bath at 90 °C until the volume was reduced to 40 mL. The concentrate was shaken thoroughly, then 20 mL of diethyl ether was added and transferred to a separating funnel. The upper ether layer was discarded, and the aqueous layer was collected in a beaker. This aqueous layer was returned to the funnel, 60 mL of n-butanol was added, and the mixture was vigorously shaken. The upper n-butanol layer was retained, and the lower aqueous layer was discarded. The n-butanol layer was washed twice with 10 mL of 5% aqueous sodium chloride. Finally, the solution was evaporated in a water bath and dried in an oven at 40 °C until a constant weight was obtained[20].

$$\text{Saponin } (\%) = \frac{\text{Weight of dried residue}}{\text{Weight of original sample}} \text{x } 100 \quad (1)$$

Quantification of alkaloids

A 10% ethanol-ethyl acetate solution (200 mL) was mixed with 5 g of the plant extract, covered, and left to stand for four hours. The mixture was filtered, and the filtrate was concentrated in a water bath until the volume was reduced to 25% of the original. Concentrated NH_4OH was then added and stirred until precipitation occurred.

The precipitate was washed with dilute NH_4OH, filtered again, dried, and weighed[22]. The alkaloid content was calculated using the following formula:

$$\text{Alkaloid } (\%) = \frac{\text{Weight of precipitate}}{\text{Weight of original sample}} \text{x } 100 \quad (2)$$

Quantification of flavonoid

A 10 g plant extract was mixed separately with 100 mL of 80% ethanol and ethyl acetate solutions for three days. The mixture was filtered using Whatman filter paper, and the filtrate was transferred to a crucible and dried in a water bath. The flavonoid content was determined by weighing the dried residue[20].

$$\text{Flavonoid } (\%) = \frac{\text{Weight of dried sample}}{\text{Weight of original sample}} \text{x } 100 \quad (3)$$

Quantification of tannins

The total tannin content was determined using the Folin-Ciocalteu method. A 0.1 mL aliquot of the plant extract was mixed with 7.5 mL of water, followed by 0.5 mL of Folin's reagent. Then, 1 mL of 35% sodium carbonate (Na_2CO_3) was added, and the volume was adjusted to 10 mL with distilled water. The mixture was shaken and allowed to stand at room temperature for 30 min. Gallic acid standards (20, 40, 60, 80, and 100 µg/mL) were prepared, and absorbance readings of both samples and standards were taken at 725 nm using a UV/Visible spectrophotometer with a blank reference. Results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extract[26].

Quantification of total phenols

Phenolic compounds were extracted by heating the sample with 50 mL of ether for 15 min. A 5 mL portion of the extract was then mixed with 2 mL of ammonium hydroxide, 5 mL of concentrated amyl alcohol, and 10 mL of distilled water in a 50 mL flask. The mixture was left to stand for 30 min for color development, and absorbance was measured at 505 nm[26].

HPLC analysis

The ethanol and ethyl acetate extracts of *Curio radicans* were analyzed using high-performance liquid chromatography (HPLC) with a Shimadzu LC-20AD HPLC system (Shimadzu, Japan). The system included a binary solvent delivery unit (LC-20AD), a Rheodyne injector with a 20-microliter sample loop, and a diode array detector (DAD; SPD-M20A). Separation of compounds was achieved through reverse-phase chromatography using a Capcell Pak C-18 column (MGII, 5 µm, 250 mm × 4.6 mm) along with an additional guard column. The mobile phase consisted of methanol, acetonitrile, and water in a ratio of 40:15:45 (v/v/v), containing 1.0% acetic acid, and was run isocratically for 30 min. Data acquisition and analysis were performed using Shimadzu LC Solution software. The DAD scanned in the wavelength range of 240 to 800 nanometers, with a flow rate set at 1 mL/min. An injection volume of 20 µl was used for both samples and standard solutions. Peaks were identified by comparing retention times and UV spectra with reference standards, while compounds were confirmed through co-injection with standard solutions[26].

Anti-microbial activity

The antimicrobial activity of *Curio radicans* was evaluated using the agar well diffusion method, with slight modifications to the procedure described in[26]. This study tested several Gram-positive and Gram-negative bacterial strains, including *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*. Additionally, antifungal activity was assessed against *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Fusarium solani*, and *Penicillium notatum*. All microbial strains were obtained from the Biotechnology Center at Abasyn University, Peshawar. Bacterial and fungal cultures were initially grown on nutrient media and incubated for 24 h. Nutrient agar was melted, cooled to around 40 °C, and poured into sterilized Petri dishes. Once solidified, wells measuring 6 mm in diameter and spaced 24 mm apart were made in the agar using a sterile steel cork borer. Actively growing bacterial cultures (4–8 h old) were evenly spread over the agar surface using sterile cotton swabs, rotating the plates during each of three streaking passes to ensure uniform distribution of the microorganisms. Each well was filled with 2 mL of the plant extract (1 mg/mL) dissolved in DMSO. For comparison, standard drugs were used as positive controls ciprofloxacin for antibacterial assays and fluconazole for antifungal assays. The plates were incubated at 37 °C for 24 h, and after incubation, the zones of inhibition around each well were measured to determine the antimicrobial activity of *C. radicans*.

Statistical analysis

Experimental data are presented as mean ± SEM. Statistical analysis was done by one-way ANOVA with Tukey's post hoc test, considering $p < 0.05$ as significant, using SPSS v20.0.

Results

Qualitative phytochemical screening of ethanolic and Ethyl acetate extracts of *Curio radicans*

The phytochemical screening of ethanolic and ethyl acetate extracts of *Curio radicans* revealed the presence of various types of secondary metabolites. The ethanolic extract showed strong positive reactions (++) for alkaloids, flavonoids, terpenoids, saponins, tannins, lipids, and phenols, indicating a high concentration of these

No.	Phytochemical Tests	Ethanolic Extract	Ethyl Acetate Extract
1	Alkaloids	(++)	(+)
2	Flavonoids	(++)	(++)
3	Terpenoids	(++)	(+)
4	Steroids	(+)	(+)
5	Carbohydrates	(+)	(-)
6	Saponins	(++)	(++)
7	Tannins	(++)	(++)
8	Glycosides	(-)	(+)
9	Lipids	(++)	(+)
10	Phenols	(++)	(++)
11	Phytosterols	(-)	(+)
12	Protein	(+)	(+)
13	Fats and Oil	(+)	(+)

Table 1. Qualitative Phytochemical Screening of Ethanolic and Ethyl Acetate Extracts of ***Curio radicans***.

Extract	Alkaloids (mg/g)	Saponins (mg/g)	Phenols (mg/g)	Flavonoids (mg/g)	Tannins (mg/g)
Ethanolic	7.76	3.53	2.50	7.60	10.32
Ethyl acetate	3.51	1.40	1.18	1.33	2.56

Table 2. Quantitative phytochemical analysis of ethanolic and Ethyl acetate extracts of *Curio radicans*.

Retention Time (min)	Compound	Molecular Formula	Molecular Weight (g/mol)	Quantity (mg/kg)
5.2	Catechin	$C_{15}H_{14}O_6$	290.26	4.55
18.4	Fumaric acid	$C_4H_4O_4$	116.07	2.15
21.3	Hydroxybenzoic acid	$C_7H_6O_3$	138.12	2.77
24.5	Caffeic acid	$C_9H_8O_4$	180.16	3.44
33.1	Salicylic acid	$C_7H_6O_3$	138.12	2.89

Table 3. HPLC analysis of ethanolic extract of *Curio radicans*.

bioactive compounds. Moderate positive responses (+) were observed for steroids, carbohydrates, proteins, and fats and oils, while glycosides and phytosterols were either absent or present in very low amounts. Similarly, the ethyl acetate extract exhibited strong positive reactions (++) for flavonoids, saponins, tannins, and phenols. Moderate positivity (+) was recorded for alkaloids, terpenoids, steroids, lipids, phytosterols, proteins, and fats and oils. Carbohydrates were not detected in this extract, while glycosides were present in small amounts. The abundance of phenolic compounds, flavonoids, and saponins in both extracts suggests that *C. radicans* may possess significant antioxidant and antimicrobial potential. The ethanolic extract was more effective in extracting alkaloids, lipids, and terpenoids, whereas the ethyl acetate extract selectively concentrated phenolic and flavonoid compounds. These findings support the potential pharmacological properties of *C. radicans* and provide a scientific basis for further phytochemical and bioactivity-guided research (Table 1).

Quantitative phytochemical analysis of ethanolic and Ethyl acetate extracts of *Curio radicans*

Quantitative phytochemical analysis of *Curio radicans* extracts revealed that the ethanolic extract contained the highest levels of all tested phytochemicals compared to the ethyl acetate extract. In the ethanolic extract, alkaloids were recorded at 7.76 mg/g, saponins at 3.53 mg/g, phenols at 2.50 mg/g, flavonoids at 7.60 mg/g, and tannins at 10.32 mg/g. In contrast, the ethyl acetate extract exhibited comparatively lower amounts, with alkaloids at 3.51 mg/g, saponins at 1.40 mg/g, phenols at 1.18 mg/g, flavonoids at 1.33 mg/g, and tannins at 2.56 mg/g. Overall, ethanolic extraction yielded a greater concentration of bioactive compounds than ethyl acetate extraction (Table 2).

HPLC analysis of ethanolic extract of *Curio radicans*

High-Performance Liquid Chromatography (HPLC) profiling of the ethanolic extract of *Curio radicans* revealed a rich spectrum of bioactive metabolites, predominantly phenolic acids and flavonoids, each identified by their distinct retention times, molecular characteristics, and quantified concentrations. The first peak at 5.2 min corresponded to Catechin ($C_{15}H_{14}O_6$; 290.26 g/mol; 4.55 mg/kg), a potent flavonoid antioxidant widely reported for its cardiovascular protective, anti-inflammatory, and antimicrobial properties. At 18.4 min, Fumaric acid

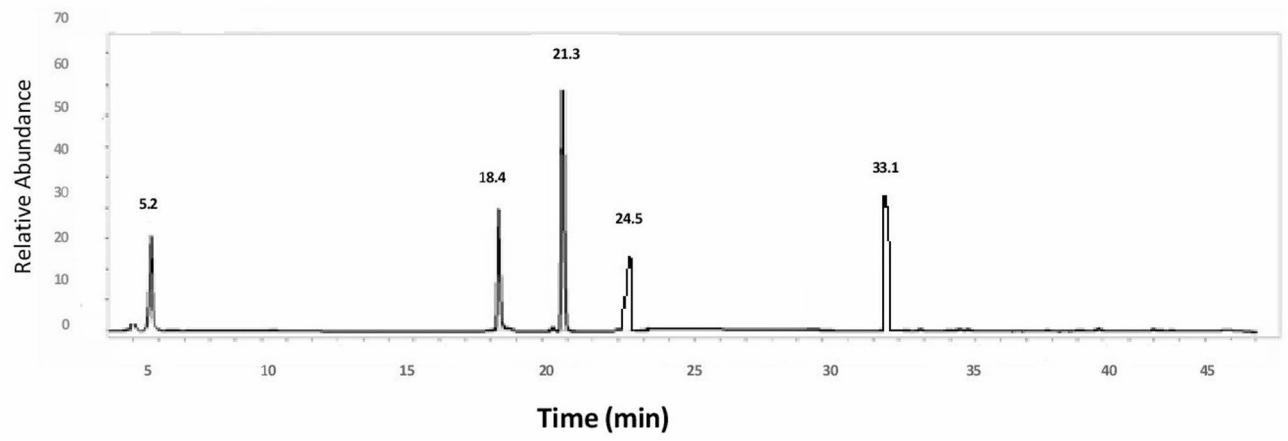

Fig. 1. HPLC chromatogram of Ethanolic extract of ***Curio radicans***

Retention Time (min)	Compound	Molecular Formula	Molecular Weight (g/mol)	Quantity (mg/kg)
6.5	Vanillin	$C_8H_8O_3$	152.15	2.89
11.3	Protocatechuic acid	$C_7H_6O_4$	154.12	2.60
20.4	Ellagic acid	$C_{14}H_6O_8$	302.19	4.10
22.4	Caffeic acid	$C_9H_8O_4$	180.16	1.45
31.5	*p*-Coumaric acid	$C_9H_8O_3$	164.16	1.21

Table 4. HPLC analysis of Ethyl acetate extract of *Curio radicans*.

($C_4H_4O_4$; 116.07 g/mol; 2.15 mg/kg) was detected, an organic acid known for its antimicrobial, anti-inflammatory, and immunomodulatory activities. The chromatogram also revealed Hydroxybenzoic acid ($C_7H_6O_3$; 138.12 g/mol; 2.77 mg/kg) at 21.3 min, a phenolic acid valued for its antioxidant and preservative potential. Caffeic acid ($C_9H_8O_4$; 180.16 g/mol; 3.44 mg/kg), detected at 24.5 min, is a well-known hydroxycinnamic acid with strong free radical scavenging, anti-inflammatory, and antimicrobial effects. The final major peak, at 33.1 min, corresponded to Salicylic acid ($C_7H_6O_3$; 138.12 g/mol; 2.89 mg/kg), a multifunctional phenolic acid with analgesic, anti-inflammatory, and antimicrobial properties. The presence of these bioactive compounds underscores the therapeutic potential of *C. radicans*, especially given the well-documented pharmacological roles of phenolic acids and flavonoids. These findings not only validate its ethnomedicinal applications but also provide a scientific basis for further pharmacological and nutraceutical research (Table 3; Figure. 1).

HPLC profiling of Ethyl acetate extract of *Curio radicans*

High-Performance Liquid Chromatography (HPLC) analysis of the ethyl acetate extract of *Curio radicans* revealed a distinct phytochemical fingerprint, characterized by five well-resolved peaks corresponding to key phenolic and aromatic compounds with established pharmacological relevance. The first eluting compound, detected at 6.5 min, was Vanillin ($C_8H_8O_3$; 152.15 g/mol; 2.89 mg/kg), a phenolic aldehyde recognized for its potent antioxidant, antimicrobial, and flavor-enhancing activities. At 11.3 min, Protocatechuic acid ($C_7H_6O_4$; 154.12 g/mol; 2.60 mg/kg) was identified, a dihydroxybenzoic acid derivative noted for its strong antioxidant, anti-inflammatory, and cytoprotective effects. A prominent peak at 20.4 min corresponded to Ellagic acid ($C_{14}H_6O_8$; 302.19 g/mol; 4.10 mg/kg), a polyphenolic compound with well-documented anticancer, antiviral, and hepatoprotective properties. At 22.4 min, Caffeic acid ($C_9H_8O_4$; 180.16 g/mol; 1.45 mg/kg) was detected, known for its free radical scavenging and anti-inflammatory actions. The final peak at 31.5 min represented p-Coumaric acid ($C_9H_8O_3$; 164.16 g/mol; 1.21 mg/kg), a hydroxycinnamic acid derivative with antimicrobial, antioxidant, and antitumor activities. The diversity of phenolic acids and aromatic compounds identified demonstrates the rich phytochemical complexity of *C. radicans*. The presence of these bioactive metabolites provides strong scientific support for the plant's ethnomedicinal applications and highlights its potential as a source of natural therapeutic agents. These results warrant further pharmacological evaluation, bioactivity-guided fractionation, and compound isolation to explore their synergistic effects and therapeutic viability (Table 4; Fig. 2).

Antibacterial activity of ethanolic and Ethyl acetate extracts of *Curio radicans*

The ethanolic and ethyl acetate extracts of *Curio radicans* were evaluated for antibacterial activity against Gram-positive and Gram-negative bacterial strains using the agar well diffusion method. Both extracts exhibited significant ($p < 0.05$) dose-dependent antibacterial activity at concentrations of 100, 200, and 300 mg/mL. Among the tested microorganisms, *Escherichia coli* was the most susceptible, showing the largest inhibition zones with both extracts. The ethanolic extract produced the highest inhibition against *E. coli* (17.40 ± 1.15 mm at 300 mg/

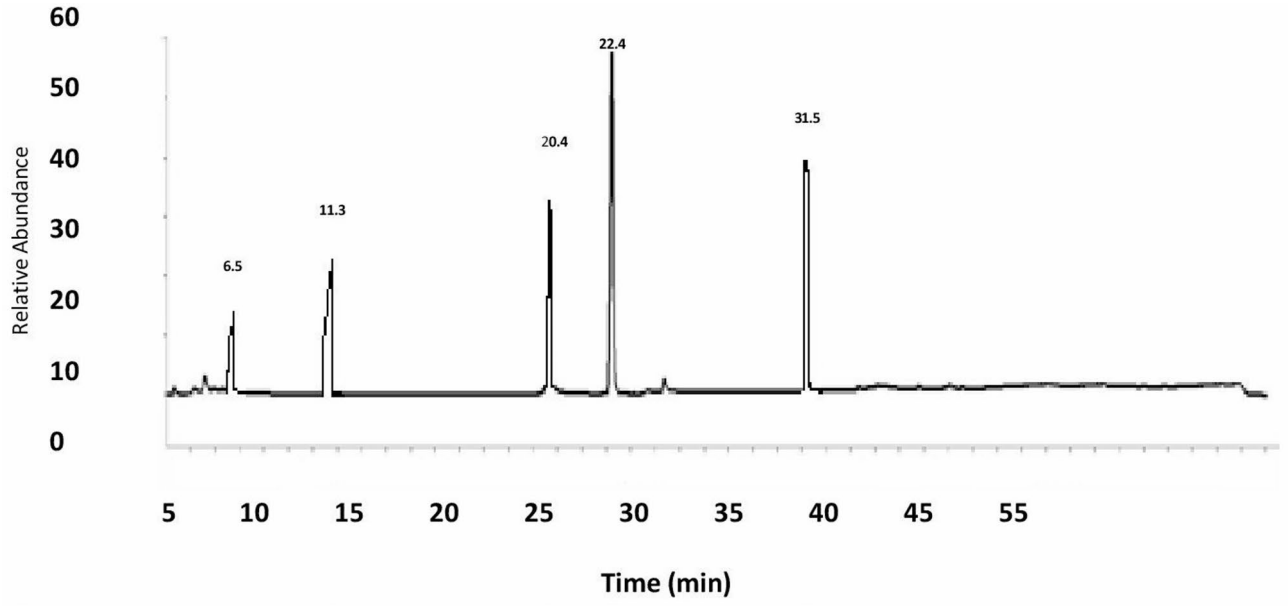

Fig. 2. HPLC chromatogram of Ethyl Acetate extract of ***Curio radicans***

Bacteria	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)
	100 mg/mL	200 mg/mL	300 mg/mL	Positive Control	Negative Control
Bacillus subtilis	8.23 ± 0.152	10.06 ± 0.152	13.70 ± 0.173	25.93 ± 0.66	0
Staphylococcus aureus	7.86 ± 0.305	9.20 ± 0.100	12.63 ± 0.305	27.03 ± 2.57	0
Staphylococcus epidermidis	9.80 ± 0.300	12.56 ± 1.59	14.30 ± 0.529	28.06 ± 1.69	0
Pseudomonas aeruginosa	10.63 ± 0.64	14.10 ± 0.20	14.63 ± 0.64	28.76 ± 0.80	0
Escherichia coli	11.96 ± 0.81	14.80 ± 1.15	17.40 ± 1.15	29.26 ± 0.49	0
Klebsiella pneumoniae	9.43 ± 0.50	13.26 ± 0.47	14.26 ± 0.56	28.80 ± 0.95	0

Table 5. Antibacterial activity of ethanolic extract of *Curio radicans*.

mL), followed by *Pseudomonas aeruginosa* (14.63 ± 0.64 mm), *Staphylococcus epidermidis* (14.30 ± 0.53 mm), and *Klebsiella pneumoniae* (14.26 ± 0.56 mm), with differences between concentrations statistically significant (ANOVA, $p < 0.05$) (Table 5). Similarly, the ethyl acetate extract demonstrated notable antibacterial activity, with *E. coli* again showing the greatest inhibition zone (17.16 ± 0.25 mm at 300 mg/mL), followed by *P. aeruginosa* (14.96 ± 0.90 mm), *S. epidermidis* (14.26 ± 0.06 mm), and *Staphylococcus aureus* (12.93 ± 0.15 mm). Statistical comparison revealed that the ethanolic extract was significantly more effective against *K. pneumoniae* and *S. aureus* ($p < 0.05$), whereas the ethyl acetate extract was equally or more effective against *E. coli* and *P. aeruginosa* ($p < 0.05$). The lowest activity in both extracts was recorded against *Bacillus subtilis*, although inhibition zones at 300 mg/mL (13.70 ± 0.17 mm for ethanolic; 13.03 ± 0.75 mm for ethyl acetate) were still significantly higher than the negative control ($p < 0.001$). In contrast, the positive control ciprofloxacin exhibited considerably larger zones of inhibition, ranging from 24.26 ± 0.80 mm to 29.93 ± 0.49 mm. These results confirm that *C. radicans* extracts possess statistically significant broad-spectrum antibacterial activity, supporting its traditional use in microbial infection management and providing a basis for further phytochemical and pharmacological investigations (Table 6).

Antifungal activity of *Curio radicans* of ethanolic and Ethyl acetate extracts

The antifungal activity of *Curio radicans* was evaluated using ethanolic and ethyl acetate extracts against six pathogenic fungal strains: *Candida albicans*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Fusarium solani*, *Aspergillus niger*, and *Penicillium notatum*. Both extracts exhibited a statistically significant ($p < 0.05$) dose-dependent increase in antifungal activity at concentrations of 100, 200, and 300 mg/mL. Among all tested fungi, *A. niger* was the most susceptible, with inhibition zones of 14.63 ± 0.15 mm for the ethanolic extract and 15.27 ± 0.39 mm for the ethyl acetate extract at 300 mg/mL, both significantly higher than at lower concentrations (ANOVA, $p < 0.05$) (Tables 7 and 8). Similarly, *P. notatum* and *F. solani* also showed notable sensitivity, with inhibition zones of 12.60 ± 1.04 mm and 12.20 ± 0.10 mm for the ethanolic extract, and 13.10 ± 0.16 mm and 13.67 ± 0.74 mm for the ethyl acetate extract, respectively, with statistically significant differences between the two extracts (t-test, $p < 0.05$). In contrast, *C. albicans* showed the least sensitivity to both extracts, with differences between concentrations not reaching statistical significance ($p > 0.05$). The positive control fluconazole exhibited significantly larger zones of

Bacteria	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)
	100 mg/mL	200 mg/mL	300 mg/mL	Positive Control	Negative Control
Bacillus subtilis	6.60 ± 0.88	9.43 ± 0.68	13.03 ± 0.75	24.26 ± 0.80	0
Staphylococcus aureus	7.63 ± 0.64	9.46 ± 0.15	12.93 ± 0.15	27.03 ± 2.57	0
Staphylococcus epidermidis	9.36 ± 0.92	13.03 ± 0.11	14.26 ± 0.057	27.40 ± 1.90	0
Pseudomonas aeruginosa	10.93 ± 0.15	13.43 ± 0.41	14.96 ± 0.90	29.10 ± 0.72	0
Escherichia coli	13.30 ± 0.65	15.96 ± 0.11	17.16 ± 0.25	29.93 ± 0.49	0
Klebsiella pneumoniae	8.83 ± 0.30	12.43 ± 0.57	13.73 ± 0.81	28.80 ± 0.95	0

Table 6. Antibacterial activity of Ethyl acetate extract of *Curio radicans*.

Fungi	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)
	100 mg/mL	200 mg/mL	300 mg/mL	Positive Control	Negative Control
Candida albicans	6.80 ± 0.30	7.86 ± 0.20	9.46 ± 0.55	27.26 ± 1.15	0
Aspergillus flavus	6.73 ± 0.47	7.70 ± 0.52	10.16 ± 0.30	27.50 ± 1.21	0
Aspergillus fumigatus	7.76 ± 0.35	9.46 ± 0.55	10.70 ± 0.52	28.40 ± 1.01	0
Fusarium solani	7.03 ± 0.20	9.83 ± 0.30	12.20 ± 0.10	27.76 ± 0.80	0
Aspergillus niger	7.70 ± 0.10	12.20 ± 1.30	14.63 ± 0.15	29.43 ± 0.83	0
Penicillium notatum	6.56 ± 0.40	10.63 ± 0.64	12.60 ± 1.04	29.56 ± 0.61	0

Table 7. Antifungal activity of *curio radicans* of ethanolic extract.

Fungi	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)
	100 mg/mL	200 mg/mL	300 mg/mL	Positive Control	Negative Control
Candida albicans	5.37 ± 0.52	7.07 ± 0.12	9.87 ± 0.05	26.27 ± 1.80	0
Aspergillus flavus	5.93 ± 0.12	7.73 ± 0.39	10.13 ± 0.17	26.83 ± 1.16	0
Aspergillus fumigatus	6.70 ± 0.08	8.83 ± 0.21	10.37 ± 0.52	27.73 ± 0.56	0
Fusarium solani	8.50 ± 0.42	11.17 ± 0.57	13.67 ± 0.74	26.10 ± 0.59	0
Aspergillus niger	9.50 ± 0.43	13.97 ± 0.09	15.27 ± 0.39	28.27 ± 1.28	0
Penicillium notatum	6.57 ± 1.11	11.10 ± 0.82	13.10 ± 0.16	29.80 ± 0.78	0

Table 8. Antifungal activity of *curio radicans* of Ethyl acetate extract.

inhibition (26.10–29.80 mm, $p < 0.001$), confirming the validity of the assay, while the negative control showed no activity. Overall, the ethyl acetate extract was statistically more effective than the ethanolic extract ($p < 0.05$), particularly against *A. niger*, suggesting that non-polar or moderately polar bioactive compounds in the ethyl acetate fraction contribute more substantially to the antifungal properties of *C. radicans*.

MIC values of antibacterial activity of ethanolic extract of *curio radicans*

The MIC values of ethanolic and ethyl acetate extracts of *Curio radicans* ranged from 2 to 6 mg/mL. The lowest MIC values (2 mg/mL) were shown by the ethyl acetate extract against *Staphylococcus aureus* (2.0 ± 0.041) and by the ethanolic extract against *Pseudomonas aeruginosa* (2.5 ± 0.061). The values of MIC attained from this research study showed that the ethanolic extract of *Curio radicans* was more potent against *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*, while the ethyl acetate extract exhibited higher activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (Table 9).

MIC values of antifungal activity of ethanolic and Ethyl acetate extracts of *curio radicans*

The MIC values of ethanolic and ethyl acetate extracts of *Curio radicans* ranged from 2 to 6 mg/mL. The lowest MIC values (2 mg/mL) were shown by the ethanolic extract against *Aspergillus flavus* (2.40 ± 0.05) and *Aspergillus fumigatus* (2.50 ± 0.03), while the ethyl acetate extract demonstrated potent inhibition against *Fusarium solani* (3.60 ± 0.05) and *Aspergillus niger* (3.90 ± 0.01). The values of MIC attained from this research study showed that both ethanolic and ethyl acetate extracts of *Curio radicans* were more effective against *Aspergillus niger*, *Penicillium notatum*, and *Candida albicans* (Table 10).

Discussion

Plants produce secondary metabolites that function as defense mechanisms against diseases and play an essential role in safeguarding human health from various illnesses. Phytochemicals are classified into six primary groups according to chemotaxonomy: carbohydrates, lipids, phenolics, terpenoids, alkaloids, and other nitrogen-

Bacteria	MIC values (mg/mL) 2 mg/mL	MIC values (mg/mL) 2 mg/mL
	Ethanol	Ethyl acetate
Bacillus subtilis	2.8 ± 0.030	3.1 ± 0.034
Staphylococcus aureus	3.3 ± 0.021	2.0 ± 0.041
Staphylococcus epidermidis	2.9 ± 0.040	2.7 ± 0.054
Pseudomonas aeruginosa	2.5 ± 0.061	2.9 ± 0.031
Escherichia coli	3.7 ± 0.028	2.7 ± 0.019
Klebsiella pneumoniae	3.1 ± 0.030	3.1 ± 0.040

Table 9. MIC values of antibacterial activity of ethanolic extract of *curio radicans*. MIC minimum inhibitory concentration. The values represent mean ± standard deviation.

Fungi	MIC values (mg/mL) 2 mg/mL	MIC values (mg/mL) 2 mg/mL
	Ethanol	Ethyl acetate
Candida albicans	3.00 ± 0.03	3.20 ± 0.01
Aspergillus flavus	2.40 ± 0.05	2.90 ± 0.06
Aspergillus fumigatus	2.50 ± 0.03	3.00 ± 0.09
Fusarium solani	2.60 ± 0.04	3.60 ± 0.05
Aspergillus niger	3.80 ± 0.02	3.90 ± 0.01
Penicillium notatum	3.70 ± 0.02	3.80 ± 0.01

Table 10. MIC values of antifungal activity of ethanolic and Ethyle acetate extracts of *curio radicans*. MIC minimum inhibitory concentration. The values represent mean ± standard deviation.

containing compounds. These bioactive substances serve as natural agents against numerous pathogens, including carcinogens. In recent years, the study and evaluation of phytoconstituents have become a major focus in medicinal plant research[27,28]. Medicinal plants are abundant sources of natural phytochemicals such as alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, resins, cardiac glycosides, coumarins, and phenolic compounds, all of which possess diverse biological activities[29].

Plant compounds are categorized as secondary metabolites because they are not vital for the immediate survival of the plants that produce them. These chemicals are distributed throughout different parts of the plant, including the bark, leaves, rhizomes, flowers, fruits, and berries. Indeed, active compounds can be present in any part of a plant. Together with nutrients and fibers, these molecules create a robust defense system that helps protect against various diseases and environmental stresses. Among the most significant bioactive phytochemicals are alkaloids, terpenoids, tannins, saponins, and phenolic compounds[30].

Phytochemical screening of ethanolic and ethyl acetate extracts of *Curio radicans* revealed a variety of secondary metabolites. The ethanolic extract showed strong positivity (++) for alkaloids, flavonoids, terpenoids, saponins, tannins, lipids, and phenols, while steroids, carbohydrates, proteins, and fats/oils showed moderate presence (+). Glycosides and phytosterols were minimal or absent. The ethyl acetate extract showed strong reactions (++) for flavonoids, saponins, tannins, and phenols, with moderate presence (+) of alkaloids, terpenoids, steroids, lipids, proteins, phytosterols, and fats/oils. Carbohydrates were absent, and glycosides were present in low amounts. These results suggest notable antioxidant and antimicrobial potential, with ethanolic extract being richer in alkaloids, lipids, and terpenoids, and ethyl acetate extract concentrating phenolics and flavonoids. These results are consistent with a previous study that reported similar findings in the Qualitative and quantitative evaluation of phytochemical constituents of *Ageratum conyzoides* L. (Asteraceae)[31].

Plants have been used in herbal medicine for thousands of years, and traditional healers around the world rely on them. In India alone, there are about 8,000 types of medicinal herbs, which make up approximately 50% of all flowering plant species[32]. In recent times, many researchers have focused on the processing of SF (*Paeoniae Alba Radix*) using various analytical methods. High-performance liquid chromatography (HPLC) tandem mass spectrometry has been successfully used to identify SF in complex mixtures[33]. Furthermore, ultra-high-performance liquid chromatography-time of flight tandem mass spectrometry, combined with principal component analysis and orthogonal partial least squares discriminant analysis, has been employed to study the differences in *Lilii Bulbus* before and after SF processing. The potential adverse effects of SF processing have also been examined through ultra-performance liquid chromatography coupled with evaporative light scattering detection and analysis of major alkaloids[34].

HPLC analysis of the ethanolic extract of *Curio radicans* identified five major bioactive compounds based on retention times and molecular weights. Catechin (5.2 min), a flavonoid with antioxidant activity, was the earliest detected compound. Fumaric acid (18.4 min) showed antimicrobial and anti-inflammatory potential. Hydroxybenzoic acid (21.3 min) and Caffeic acid (24.5 min) were noted for their antioxidant and anti-inflammatory properties. Salicylic acid (33.1 min) was identified as a phenolic acid with known antimicrobial

and anti-inflammatory effects. The presence of these phenolic acids and flavonoids underscores the therapeutic potential of *C. radicans* and supports its traditional medicinal use. These findings are consistent with a previous report that demonstrated similar results in the HPLC analysis and biological activities of *Rhanterium adpressum* extracts (Asteraceae)[35]. Catechin is a polyphenol that possess antimicrobial potential, acting mainly against Gram-positive bacteria such as *Staphylococcus aureus*. Its antimicrobial effect is attributed to binding with pathogen cell proteins, which disrupts their function, increases membrane permeability, and causes leakage of cellular contents, ultimately leading to growth inhibition or cell death[36]. Fumaric acid exhibit antimicrobial significance primarily by lowering intracellular pH, disrupting microbial metabolism, and interfering with enzyme function, ultimately inhibiting growth and survival of pathogens[37]. Caffeic acid and its derivatives exert antimicrobial activity by disrupting microbial membranes, generating oxidative stress, and inhibiting key enzymes, often enhanced through nanoformulations or synergistic combinations with antibiotics[38].

HPLC analysis of the ethyl acetate extract of *Curio radicans* identified five key bioactive compounds with known pharmacological properties. Vanillin (6.5 min) showed antioxidant and antimicrobial activities. Protocatechuic acid (11.3 min) exhibited antioxidant, anti-inflammatory, and cytoprotective effects. Ellagic acid (20.4 min) was noted for anti-carcinogenic and antiviral properties. Caffeic acid (22.4 min) demonstrated strong antioxidant and anti-inflammatory action, while Coumaric acid (31.5 min) possessed antimicrobial, antioxidant, and anticancer activities. These results highlight the rich phytochemical profile and therapeutic potential of *C. radicans* and are in agreement with previous findings[39]which reported comparable HPLC analysis, phenolic compound composition, and bioactivities of the ethanolic extract from the flowers of Moroccan *Anacyclus clavatus*.

Ellagic acid exhibits antimicrobial effect by damaging microbial cell membranes, chelating metal ions, inhibiting essential enzymes, and inducing oxidative stress, leading to growth inhibition and cell death[40–42].

In recent years, the rising demand for plant-derived antibacterial agents has offered a promising approach to address the need for new therapeutics while reducing dependence on conventional antibiotics. This growing interest has motivated researchers to investigate novel bioactive compounds capable of combating microbial resistance[43]. As many of the medicinal properties of plants are still unexplored, there is increasing focus on discovering new antimicrobial molecules that may function either alone or synergistically with existing antibiotics to improve their efficacy[44].

The antimicrobial activity of plant extracts of *C. radicans* might be due to several phytochemicals such as alkaloids, flavonoids, terpenoids, saponins, tannins, lipids, and phenols, while steroids, carbohydrates, proteins, and fats/oils. Phenols affect the function of the cytoplasmic membrane, disturbing the metabolism of energy, and thus affecting the synthesis of nucleic acids[45]. Terpenoids and alkaloids interact with enzymes and proteins of the microbial cell membrane causing its disruption to disperse a flux of protons towards the cell exterior which induces cell death or may inhibit enzymes necessary for amino acids biosynthesis[46]. Flavonoids have been shown to inhibit bacterial DNA polymerase, RNA polymerase, Reverse Transcriptase, and Telomerase[47]. The saponins decrease surface tension causing an increase in permeability or leakage of cells, resulting in the discharge of intracellular compounds[48].

The ethanolic and ethyl acetate extracts of *Curio radicans* were tested for antibacterial activity against Gram-positive and Gram-negative bacteria using the agar well diffusion method at 100, 200, and 300 mg/mL. Both extracts showed dose-dependent, broad-spectrum antibacterial effects. *Escherichia coli* was the most sensitive, with the highest inhibition zones observed for the ethanolic (17.40 ± 1.15 mm) and ethyl acetate (17.16 ± 0.25 mm) extracts at 300 mg/mL. Other notable results included inhibition of *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. The lowest activity was against *Bacillus subtilis*, though significant zones were still noted. Positive controls showed higher inhibition, while negative controls had no effect. Overall, the ethanolic extract was slightly more effective against *K. pneumoniae* and *S. aureus*, while the ethyl acetate extract performed better against *E. coli* and *P. aeruginosa*. These results support the traditional use of *C. radicans* for treating microbial infections and are consistent with previous findings[49]which reported similar antimicrobial potential in *Crassocephalum bauchiense*.

Bioactive polyphenols present in many plants have shown antimicrobial activity by disrupting the functions of microbial organisms[50,51]. These compounds penetrate microbial cells, damage their cell membranes through hydrophobic interactions, and inhibit crucial enzymatic processes like DNA gyrase activity and RNA synthesis. Consequently, these pathogens are unable to survive or interfere with human cellular functions. Epidemiological studies have also indicated that a diet high in plant polyphenols is associated with a lower risk of chronic diseases such as cancer, cardiovascular disease, diabetes, osteoporosis, and neurological disorders[8,52].

The ethanolic and ethyl acetate extracts of *Curio radicans* were tested for antifungal activity against six pathogenic fungi. Both extracts showed dose-dependent effects, with the ethyl acetate extract generally more potent. *Aspergillus niger* was the most sensitive fungus, showing inhibition zones of 14.63 ± 0.15 mm (ethanolic) and 15.27 ± 0.39 mm (ethyl acetate) at 300 mg/mL. *Penicillium notatum* and *Fusarium solani* also showed notable sensitivity. *Candida albicans* was the least affected. Positive controls confirmed assay reliability, while negative controls showed no activity. The greater efficacy of the ethyl acetate extract suggests that moderately polar bioactive compounds may contribute significantly to *C. radicans*' antifungal properties. These findings agree with previous work[53]which reported similar antifungal activity in extracts of *Avicennia marina*.

Conclusion

The findings of this study demonstrate that *Curio radicans* possesses a rich profile of bioactive phytochemicals, with the ethanolic extract yielding higher concentrations of alkaloids, flavonoids, tannins, and other metabolites, while the ethyl acetate extract exhibited notable phenolic enrichment. HPLC profiling confirmed the presence of diverse antimicrobial compounds. Both extracts showed significant antibacterial and antifungal activities, supporting the plant's traditional medicinal applications. These results highlight *C. radicans* as a promising

natural source of therapeutic agents and warrant further bioactivity-guided isolation and pharmacological evaluation to explore its potential in drug development.

Data availability

All the data is available in the main manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

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