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GC–MS-based phytochemical characterization, antioxidant, anticancer and antibacterial activities of *Corallocarpus epigaeus* (Rottler) C.B. Clarke rhizome extracts

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Abstract
Introduction: *Corallocarpus epigaeus* (Rottler) C.B. Clarke is a medicinal plant traditionally used for the treatment of various ailments. However, scientific evidence regarding its phytochemical composition and biological activities remains limited. The present study aimed to investigate the phytochemical constituents, antioxidant, anticancer, and antibacterial activities of methanol, acetone, and aqueous extracts of *C. epigaeus* rhizomes.
Methods: Rhizome extracts were prepared using methanol, acetone, and water. Total phenolic content (TPC) and total flavonoid content (TFC) were determined spectrophotometrically. The phytochemical profile of the methanol extract was analysed using gas chromatography–mass spectrometry (GC–MS). Antioxidant activity was evaluated by DPPH, hydrogen peroxide (H_2O_2), nitric oxide (NO) scavenging, and reducing power assays. Anticancer activity was assessed against LoVo and HCT-116 human colon cancer cell lines using MTT, Hoechst 33342 staining, lactate dehydrogenase (LDH) cytotoxicity, and wound healing assays. Antibacterial activity was determined by the agar well diffusion method against selected Gram-positive and Gram-negative bacterial strains.
Results: The aqueous extract yielded the highest extraction efficiency (13.07 ± 0.24%), whereas the methanol extract contained the highest total phenolic content (282.1 ± 11.2 mg GAE/g) and the acetone extract exhibited the highest flavonoid content (7.1 ± 0.9 mg QE/g). GC–MS analysis identified thirteen major compounds, with germacrene-D (22.01%), palmitone (16.92%), and trans-caryophyllene (12.12%) as the predominant constituents. The acetone extract showed the strongest DPPH radical scavenging activity (IC_{50} = 33.9 ± 4.8 µg/mL), while the methanol extract demonstrated superior nitric oxide scavenging activity (IC_{50} = 12 ± 4.2 µg/mL). All extracts exhibited significant reducing power and concentration-dependent antioxidant activity. *In -vitro* anticancer assays revealed marked inhibition of LoVo and HCT-116 cell proliferation, induction of apoptotic nuclear changes, increased LDH-mediated cytotoxicity, and suppression of cell migration. Furthermore, the methanol and acetone extracts displayed broad-spectrum antibacterial activity, particularly against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
Conclusion: The findings demonstrate that *Corallocarpus epigaeus* rhizomes are rich in bioactive phytochemicals possessing potent antioxidant, anticancer, and antibacterial activities. The observed biological effects may be attributed to the synergistic action of phenolic compounds and terpenoid constituents identified by GC–MS analysis. These results support the potential use of *C. epigaeus* as a promising source of natural therapeutic agents and warrant further investigation through mechanistic and *In -vivo* studies.

Keywords: *Corallocarpus epigaeus*, rhizome, GC–MS, antioxidant activity, anticancer activity, colon cancer, LoVo, HCT-116, antibacterial activity, phytochemicals

Introduction

Oxidative stress has emerged as one of the most extensively investigated biological phenomena due to its significant role in the pathogenesis of numerous human diseases, including inflammatory disorders, cardiovascular diseases, diabetes, neurodegenerative conditions, and cancer. Oxidative stress occurs when the production of reactive oxygen and nitrogen species (RONS) exceeds the cellular antioxidant defence system, leading to damage of biomolecules such as lipids, proteins, and nucleic acids. Persistent oxidative stress disrupts cellular homeostasis, promotes chronic inflammation, and contributes to the initiation and progression of various pathological conditions, particularly cancer. Numerous studies have demonstrated that excessive generation of RONS is closely associated with carcinogenesis, tumour growth, metastasis, and resistance to conventional therapies.

Cancer remains one of the leading causes of mortality worldwide and represents a major public health challenge. Although treatment modalities such as chemotherapy, radiotherapy, surgery, and targeted therapies have significantly improved patient survival rates, their clinical effectiveness is often limited by severe side effects, high treatment costs, and the development of drug resistance. Consequently, there is increasing interest in identifying novel therapeutic agents from natural sources that can act as effective anticancer compounds with minimal toxicity. Medicinal plants have historically served as valuable reservoirs of bioactive molecules and continue to contribute significantly to modern drug discovery. Plant-derived phytochemicals such as flavonoids, alkaloids, terpenoids, phenolics, and glycosides exhibit diverse pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties.

The emergence and rapid spread of antimicrobial resistance have become a serious global health concern. Excessive and inappropriate use of antibiotics has accelerated the evolution of resistant microbial strains, reducing the effectiveness of conventional antimicrobial therapies. The World Health Organization has highlighted antimicrobial resistance as one of the major threats to global health and has emphasized the urgent need for the discovery of new antimicrobial agents. Natural products derived from medicinal plants offer promising alternatives because they contain multiple bioactive compounds capable of targeting microorganisms through different mechanisms, thereby reducing the likelihood of resistance development. Consequently, considerable attention has been directed toward exploring medicinal plants for their antimicrobial potential.

Corallocarpus epigaeus (Rottler) C.B. Clarke, a perennial medicinal climber belonging to the family Cucurbitaceae, is widely distributed in India, Sri Lanka, and other tropical regions of Asia. The plant is traditionally used in various indigenous systems of medicine for the treatment of skin diseases, inflammation, rheumatism, ulcers, fever, snakebite, diabetes, and other ailments. The tuberous roots and other plant parts are known to contain a variety of secondary metabolites, including flavonoids, alkaloids, terpenoids, phenolic compounds, tannins, and glycosides, which are believed to contribute to its therapeutic properties. Previous pharmacological investigations have reported anti-inflammatory, antioxidant, antimicrobial, antidiabetic, hepatoprotective, and cytotoxic activities of extracts obtained from different parts of the plant.

Despite its traditional medicinal importance and reported biological activities, comprehensive studies evaluating the phytochemical composition and biological properties of Corallocarpus epigaeus remain limited. In particular, information regarding its antioxidant, antibacterial, and anticancer potential is still insufficient. Therefore, the present study was undertaken to investigate the phytochemical constituents and evaluate the antioxidant, antibacterial, and anticancer activities of different solvent extracts of Corallocarpus epigaeus. The findings of this study may contribute to the identification of novel bioactive compounds and support the potential use of this medicinal plant in the development of natural therapeutic agents.

Plant Material

Fresh tubers of *Corallocarpus epigaeus* (Rottler) C.B. Clarke were collected from the region of Paali village, Ulundurpet Taluk, Kallakurichi District, Tamil Nadu, India, during June 2026. The plant material was identified and authenticated by a qualified taxonomist at The Rapinat Herbarium, St. Joseph's College (Autonomous), Trichirappalli, Tamil Nadu, India. A voucher specimen S. No:1007 was deposited in The Rapinat Herbarium, Trichirappalli for future reference.

Extraction of Plant Material:

The collected tubers were thoroughly washed with running tap water to remove adhering soil and other impurities, followed by rinsing with distilled water. The cleaned plant material was cut into small pieces and shade-dried at room temperature (25–30 °C) for 10–14 days until a constant weight was obtained. The dried tubers were then pulverized into a fine powder using a mechanical grinder and passed through a fine mesh sieve to ensure uniform particle size. The powdered material was stored in airtight containers at 4 °C until further extraction and phytochemical analyses were performed.

The dried tuber powder of *Corallocarpus epigaeus* (Rottler) C.B. Clarke was subjected to extraction using three solvents of varying polarity, namely methanol, acetone, and distilled water. For the preparation of methanol and acetone extracts, the powdered material was separately soaked in methanol and acetone at a ratio of 1:10 (w/v) and allowed to macerate for 48 h at room temperature with intermittent shaking. The resulting mixtures were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator at temperatures below 40 °C to obtain crude extracts.

For the aqueous extraction, the powdered tuber material was mixed with distilled water (1:10, w/v) and macerated for 48 h at room temperature. The extract was centrifuged at 10,000 rpm for 10 min to remove insoluble debris. The supernatant was subsequently filtered through Whatman No. 1 filter paper and freeze-dried using a laboratory lyophilizer to obtain the aqueous crude extract.

The dried methanol, acetone, and aqueous extracts were weighed individually to determine extraction yield (% w/w) and transferred into sterile amber-colour containers. All extracts were stored at 4 °C until further phytochemical screening, GC–MS analysis, antioxidant assays, antibacterial studies, and anticancer investigations. Prior to experimental use, each extract was reconstituted in its respective extraction solvent to obtain the desired concentrations.

Materials and Methods

Total Phenolic Content

The total phenolic content (TPC) of *Corallocarpus epigaeus* (Rottler) C.B. Clarke tuber extracts was determined using the Folin–Ciocalteu colorimetric method with slight modifications. Briefly, 0.3 mL of each extract solution (80 µg/mL) was mixed with 1.5 mL of Folin–Ciocalteu reagent previously diluted ten-fold with distilled water. After 5 min of incubation, 1.2 mL of sodium carbonate solution (7.5%, w/v) was added, and the reaction mixture was thoroughly mixed. The resulting solution was incubated at room temperature for 30 min in the dark to allow complete colour development.

The absorbance of the reaction mixture was measured at 765 nm using a UV–Visible spectrophotometer against an appropriate blank. Gallic acid was used as the reference standard for the preparation of the calibration curve, and the total phenolic content was calculated from the regression equation obtained from the standard curve. The results were expressed as milligrams of Gallic acid equivalents per gram of dry extract (mg GAE/g DW). All measurements were performed in triplicate, and the results were expressed as mean ± standard deviation.

Total Flavonoid Content

The total flavonoid content (TFC) of *Corallocarpus epigaeus* (Rottler) C.B. Clarke tuber extracts was quantified using the aluminium chloride colorimetric method described by Loganayaki *et al*. with slight modifications. Briefly, 0.25 mL of each extract solution (80 µg/mL) was mixed with 1.0 mL of distilled water, followed by the addition of 0.075 mL of 5% sodium nitrite ($NaNO_2$) solution. After incubation for 5 min at room temperature, 0.15 mL of 10% aluminium

chloride ($AlCl_3$) solution was added and the mixture was allowed to stand for an additional 6 min. Subsequently, 0.5 mL of 4% sodium hydroxide (NaOH) solution was added, and the final volume was adjusted to 5 mL using distilled water. The reaction mixture was thoroughly mixed and incubated for 15 min at room temperature.

The absorbance was measured at 415 nm using a UV–Visible spectrophotometer against a reagent blank. Quercetin was used as the reference standard, and a calibration curve was constructed using different concentrations of quercetin. The total flavonoid content was expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g DW). All analyses were carried out in triplicate, and the results were reported as mean ± standard deviation.

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The phytochemical constituents present in the methanol extract of *Corallocarpus epigaeus* tubers were identified using Gas Chromatography–Mass Spectrometry (GC–MS) analysis. The analysis was performed using a Thermo Trace GC Ultra gas chromatograph coupled with a TSQ Quantum triple quadruple mass spectrometer. Electron ionization was carried out at 70 eV, and mass spectra were recorded in full-scan mode over a mass range of 40–500 m/z.

Chromatographic separation was achieved using a Thermo TR-5MS fused silica capillary column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness) coated with 5% phenyl polysilphenylene-siloxane as the stationary phase. The oven temperature program was initiated at 40 °C and gradually increased to 300 °C at a rate of 6 °C/min. The injector temperature was maintained at 250 °C.

Helium (99.999% purity) was employed as the carrier gas at a constant flow rate of 1.0 mL/min with a split ratio of 25:1. The transfer line temperature was maintained at 250 °C. Identification of the separated compounds was accomplished by comparing the obtained mass spectra with those available in the National Institute of Standards and Technology (NIST) library, Wiley mass spectral database, and previously published literature reports. The relative abundance of individual compounds was determined from their respective peak areas in the total ion chromatogram.

Antioxidant Activity

DPPH Free Radical Scavenging Assay

The free radical scavenging potential of *Corallocarpus epigaeus* (Rottler) C.B. Clarke tuber extracts was evaluated using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. This method is based on the reduction of the stable purple-colour DPPH radical to a yellow-colour diphenylpicrylhydrazine compound in the presence of antioxidant molecules.

Briefly, 1.0 mL of DPPH solution (0.1 mm prepared in methanol) was mixed with 1.0 mL of plant extract at different concentrations ranging from 10 to 100 µg/mL. A control solution containing DPPH and solvent without extract was prepared simultaneously. L-ascorbic acid, at concentrations between 2 and 100 µg/mL, was used as the reference antioxidant standard. All reaction mixtures were prepared in triplicate and incubated in darkness at room temperature for 30 min to ensure complete reaction.

Following incubation, the absorbance of each mixture was recorded at 517 nm using a UV–Visible spectrophotometer. A decrease in absorbance indicated the ability of the extract to neutralize DPPH free radicals. The percentage inhibition of DPPH radicals was calculated using the following equation:

DPPH Radical Scavenging Activity (%) = $[(A_0 - A_1) / A_0] \times 100$

Where:

- **A_0** = Absorbance of the control reaction
- **A_1** = Absorbance of the extract or standard sample

The concentration required to inhibit 50% of the DPPH radicals (IC_{50}) was determined from the dose–response curve and used as an indicator of antioxidant potency.

Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide (H_2O_2) scavenging capacity of *Corallocarpus epigaeus* (Rottler) C.B. Clarke tuber extracts was assessed spectrophotometrically following a modified protocol based on the method of Ruch *et al*. Hydrogen peroxide is a weak oxidizing agent that can penetrate biological membranes and generate highly reactive hydroxyl radicals, thereby contributing to oxidative stress.

For the assay, 0.1 mL of each extract at different concentrations (0.1–1.0 mg/mL) was mixed with 0.6 mL of 40 mm hydrogen peroxide solution prepared in phosphate buffer (pH 7.4). The reaction mixtures were gently vortexes and allowed to stand at room temperature for 10 min. A control solution containing hydrogen peroxide and phosphate buffer without plant extract was prepared under identical conditions. L-ascorbic acid (0.1–1.0 mg/mL) served as the positive control.

After incubation, the absorbance of the reaction mixtures was recorded at 230 nm using a UV–Visible spectrophotometer, with phosphate buffer used as the blank. The decrease in absorbance relative to the control indicated the ability of the extract to neutralize hydrogen peroxide.

The percentage scavenging activity was calculated using the following formula:

Hydrogen Peroxide Scavenging Activity (%) = $[(A_0 - A_1) / A_0] \times 100$

Where:

- **A_0** = Absorbance of the control
- **A_1** = Absorbance of the test sample or standard

The IC_{50} value, defined as the concentration required to scavenge 50% of hydrogen peroxide radicals, was calculated from the inhibition curve and used to compare the antioxidant effectiveness of the extracts.

Nitric Oxide Scavenging Activity

The nitric oxide (NO) radical scavenging potential of *Corallocarpus epigaeus* (Rottler) C.B. Clarke tuber extracts was evaluated using the sodium nitroprusside–Griess reaction method. Under physiological conditions, sodium nitroprusside spontaneously generates nitric oxide, which reacts with oxygen to form nitrite ions. The extent of nitrite formation can be estimated using Gris reagent, and a

reduction in nitrite concentration indicates nitric oxide scavenging activity.

Briefly, 0.5 mL of 10 mm sodium nitroprusside prepared in phosphate-buffered saline (PBS, pH 7.4) was mixed with 1.0 mL of plant extract at concentrations ranging from 10 to 100 µg/mL. The reaction mixtures were incubated at 25 °C for 180 min under normal laboratory conditions. Following incubation, an equal volume of freshly prepared Gris reagent was added to each mixture. The Gris reagent consisted of 1% sulfanilamide dissolved in 5% phosphoric acid and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride prepared in distilled water.

A control containing phosphate-buffered saline instead of plant extract and a blank without sodium nitroprusside were prepared simultaneously. Gallic acid and L-ascorbic acid (10–100 µg/mL) were employed as reference antioxidant standards. After complete colour development, the absorbance was measured at 546 nm using a UV–Visible spectrophotometer.

The nitric oxide radical scavenging activity was calculated using the following equation:

Nitric Oxide Scavenging Activity (%) = $[(A_0 - A_1) / A_0] \times 100$

Where:

- **A_0** = Absorbance of the control
- **A_1** = Absorbance of the sample or standard

The concentration required to inhibit 50% of nitric oxide radicals (IC_{50}) was determined from the inhibition curve and used as a measure of the antioxidant efficiency of the extracts.

Reducing Power Assay

The electron-donating ability of *Corallocarpus epigaeus* (Rottler) C.B. Clarke tuber extracts was determined using the ferric reducing power assay with minor modifications to the method originally described by Oyaizu. The assay is based on the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) by antioxidant compounds present in the plant extracts.

Different concentrations of the extracts (0.25–1.0 mg/mL) were prepared and mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution. The reaction mixtures were incubated at 50 °C for 20 min to facilitate the reduction process. Following incubation, 2.5 mL of 10% trichloroacetic acid was added to terminate the reaction, and the mixtures were centrifuged at 650 rpm for 10 min.

After centrifugation, 2.5 mL of the clear supernatant was carefully transferred to a fresh test tube and combined with an equal volume of distilled water. Subsequently, 0.5 mL of freshly prepared 0.1% ferric chloride solution was added, and the mixtures were allowed to react for a few minutes at room temperature.

The absorbance was then recorded at 700 nm using a UV–Visible spectrophotometer. Increased absorbance values indicated greater reducing power and stronger antioxidant activity of the extracts. Butylated hydroxytoluene (BHT) and L-ascorbic acid were used as positive control standards for comparison. All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation.

Anti-proliferative Activity- Cell Culture

The human colorectal carcinoma cell lines LoVo and HCT-116 were procured from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% antibiotic solution containing penicillin and streptomycin to prevent microbial contamination.

The cultures were grown in sterile tissue culture flasks and incubated at 37 °C in a humidified incubator supplied with 5% CO_2 and 95% air. Cell morphology and growth were monitored regularly using an inverted microscope. The culture medium was renewed every 48 h to ensure adequate nutrient supply and removal of metabolic waste products.

Upon reaching approximately 80–90% confluence, the cells were detached using trypsin–EDTA solution, harvested, and subculture for further experiments. Only healthy and exponentially growing cells were used for cytotoxicity and antiproliferative assays to ensure reproducibility and reliability of the results.

Cell Viability Assay (MTT Assay)

The cytotoxic effect of *Corallocarpus epigaeus* (Rottler) C.B. Clarke tuber extracts on cancer cells was evaluated using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. This colorimetric method measures the metabolic activity of viable cells based on the conversion of MTT into insoluble purple Formosan crystals by mitochondrial dehydrogenase enzymes.

Briefly, LoVo and HCT-116 cells were seeded into 6-well culture plates at a density of 4×10^5 cells per well and allowed to attach overnight under standard culture conditions. The cells were then exposed to various concentrations of *C. epigaeus* extract (1, 10, and 100 µg/mL) for 24 h. untreated cells maintained in complete culture medium served as the control group.

Following treatment, the culture medium was carefully aspirated, and the cells were gently rinsed twice with phosphate-buffered saline (PBS) to remove residual extract. Subsequently, 100 µL of MTT solution (5 mg/mL in PBS) was added to each well, and the plates were incubated for 4 h at 37 °C to allow the formation of Formosan crystals.

At the end of incubation, the MTT solution was discarded, and the resulting purple Formosan crystals were dissolved by adding 500 µL of 0.04 N hydrochloric acid in isopropanol. The plates were gently agitated to ensure complete solubilisation of the crystals. Thereafter, 100 µL of the dissolved reaction mixture from each sample was transferred to a 96-well micro plate, and the absorbance was recorded at 550 nm using a micro plate reader.

All treatments were performed in triplicate, and the results were expressed as mean ± standard deviation. Cell viability was calculated relative to untreated control cells using the following equation:

Cell Viability (%) = (Absorbance of Treated Cells / Absorbance of Control Cells) × 100

The percentage of growth inhibition was determined by subtracting the cell viability percentage from 100. The concentration required to inhibit 50% of cell growth (IC_{50}) was estimated from the dose–response curve and used as an indicator of cytotoxic potency.

Cell Viability (%) = (Absorbance of Treated Cells / Absorbance of Negative Control Cells) × 100

Where:

- Absorbance of Treated Cells represents the optical density (OD) of cells exposed to *Corallocarpus epigaeus* extract.
- Absorbance of Negative Control Cells represents the OD of untreated cells maintained under identical experimental conditions.

The percentage of growth inhibition was calculated using the following equation:

Growth Inhibition (%) = 100 – Cell Viability (%)

Nuclear Staining Assay

The effect of *Corallocarpus epigaeus* (Rottler) C.B. Clarke tuber extracts on nuclear morphology and apoptosis induction was evaluated using Hoechst 33342 fluorescent staining. This assay enables the visualization of characteristic apoptotic features, including chromatin condensation, nuclear fragmentation, and apoptotic body formation.

Briefly, cancer cells were seeded into 6-well culture plates at a density of 3×10^5 cells per well and incubated overnight under standard culture conditions to allow cell attachment. The culture medium was then replaced with fresh medium containing different concentrations of the plant extract, and the cells were further incubated for 24 h.

Following treatment, the culture medium was removed, and the cells were gently washed twice with phosphate-buffered saline (PBS) to eliminate residual extract. The cells were subsequently fixed with chilled methanol for 15 min at room temperature. After fixation, the cells were rinsed twice with PBS and stained with Hoechst 33342 solution (2 µg/mL) for 15 min in the dark to prevent photo bleaching.

Excess stain was removed by washing the cells twice with PBS. The stained nuclei were then examined under a fluorescence microscope, and representative images were captured for morphological analysis. Nuclear changes such as chromatin condensation, fragmented nuclei, and apoptotic bodies were considered indicative of apoptosis induced by the *C. epigaeus* extract.

Cytotoxicity Assay (Lactate Dehydrogenase Release Assay

The cytotoxic potential of *Corallocarpus epigaeus* (Rottler) C.B. Clarke tuber extracts was evaluated by determining lactate dehydrogenase (LDH) release from damaged cells. LDH is a stable cytoplasmic enzyme that is released into the extracellular environment following loss of cell membrane integrity and is widely used as an indicator of cellular injury and cytotoxicity.

Briefly, LoVo and HCT-116 cells were seeded into 6-well culture plates at a density of 4×10^5 cells per well and allowed to grow for 24 h under standard incubation conditions. After overnight attachment, the existing culture medium was replaced with fresh medium containing different concentrations of *C. epigaeus* extract (1, 10, and 100 µg/mL). The treated cells were incubated for an additional 24 h.

Following treatment, aliquots of the culture supernatant were collected for LDH estimation. Approximately 10 µL of the supernatant from each sample was transferred into a 96-well micro plate, and LDH activity was measured using a commercial LDH Cytotoxicity Colorimetric Assay Kit according to the manufacturer's protocol. The enzymatic reaction was allowed to proceed under the recommended conditions, and absorbance was recorded using a micro plate reader.

The extent of cytotoxicity was expressed as a percentage and calculated using the following equation:

$\text{Cytotoxicity (\%)} = [(A_s - A_n) / (A_p - A_n)] \times 100$

Where:

- **A_s** = Absorbance of the treated sample
- **A_n** = Absorbance of the negative control (untreated cells)
- **A_p** = Absorbance of the positive control (maximum LDH release)

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation. An increase in LDH release was interpreted as enhanced membrane damage and cytotoxic activity of the plant extract.

Antibacterial Activity (Bacterial Strains and Growth Conditions)

The antibacterial efficacy of *Corallocarpus epigaeus* (Rottler) C.B. Clarke rhizome extracts was evaluated against a panel of clinically significant Gram-positive and Gram-negative bacterial pathogens. The selected test organisms included *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Salmonella typhimurium*. These bacterial strains were obtained from a recognized microbial culture collection and maintained under standard laboratory conditions.

For experimental use, each bacterial strain was inoculated into Luria–Bertani (LB) broth and incubated at 37 °C for 18–24 h to obtain actively growing cultures. The turbidity of the bacterial suspensions was adjusted to match the 0.5 McFarland standard, corresponding to approximately 1×10^8 colony-forming units (CFU)/mL. The standardized cultures were subsequently diluted with sterile physiological saline (0.9% NaCl) to obtain a final inoculum concentration of approximately 1×10^6 CFU/mL. The prepared bacterial suspensions were used immediately for antibacterial susceptibility testing of the methanol, acetone, and aqueous extracts of *C. epigaeus* rhizomes.

Agar Well Diffusion Assay

The antibacterial activity of *Corallocarpus epigaeus* (Rottler) C.B. Clarke rhizome extracts was evaluated using the agar well diffusion technique. This method was employed to determine the inhibitory effect of the plant extracts against selected bacterial pathogens. Briefly, standardized bacterial suspensions (approximately 1×10^6 CFU/mL) were evenly spread onto sterile Mueller–Hinton agar plates using sterile cotton swabs to obtain a uniform bacterial lawn. After inoculation, wells of 6 mm diameter were aseptically created in the agar medium using a sterile cork borer.

Each well was loaded with 100 µL of methanol, acetone, or aqueous extract prepared at a concentration of 50 mg/mL. Dimethyl sulfoxide (DMSO) was used as the solvent for

extract preparation. A standard antibiotic disc containing tetracycline (30 µg/disc) was included as the positive control, whereas DMSO alone served as the negative control to verify the absence of antimicrobial activity from the solvent. The inoculated plates were incubated aerobically at 37 °C for 24 h. Following incubation, the antibacterial activity of the extracts was assessed by measuring the diameter of the clear zones surrounding the wells. The inhibition zones were recorded in millimetres (mm), and larger zones were considered indicative of stronger antibacterial activity. All experiments were conducted in duplicate, and the results were expressed as mean inhibition zone diameter ± standard deviation.

Statistical Analysis

All experimental data were analysed using Statistical Package for the Social Sciences (SPSS) software (Version 21.0, IBM Corp., and USA). The results obtained from independent experiments were expressed as mean ± standard deviation (SD).

Differences among experimental groups were evaluated using one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test to compare treated groups with the corresponding control group. Statistical significance was determined at a confidence level of 95%.

A probability value ($P < 0.05$) was considered statistically significant, whereas $P < 0.01$ was regarded as highly significant. All experiments were performed in triplicate, and the statistical analyses were conducted to ensure the reliability and reproducibility of the observed results.

Results

Extraction Yield, Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Antioxidant Activity of Corallocarpus epigaeus Rhizome Extracts

The extraction yield, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities of the methanol, acetone, and aqueous extracts of *Corallocarpus epigaeus* rhizomes are presented in Table 1. Among the three extracts, the aqueous extract produced the highest extraction yield (13.07 ± 0.24%), followed by the methanol (6.96 ± 0.68%) and acetone (5.84 ± 0.33%) extracts. The greater extraction efficiency observed with water may be attributed to its ability to solubilize a wide range of polar constituents present in the rhizome.

The methanol extract exhibited the highest total phenolic content (282.1 ± 11.2 mg GAE/g extract), followed by the acetone extract (256.3 ± 12.5 mg GAE/g extract), whereas the aqueous extract contained considerably lower levels of phenolic compounds (16.9 ± 0.4 mg GAE/g extract). These findings indicate that methanol was more effective in extracting phenolic constituents from *C. epigaeus* rhizomes. In contrast, the acetone extract showed the highest total flavonoid content (7.1 ± 0.9 mg QE/g extract), which was approximately four times greater than that of the methanol extract (1.8 ± 0.1 mg QE/g extract). Only trace quantities of flavonoids were detected in the aqueous extract (0.1 ± 0.05 mg QE/g extract).

The antioxidant activity of the extracts was evaluated using DPPH, hydrogen peroxide (H_2O_2), and nitric oxide (NO) radical scavenging assays. In the DPPH assay, the acetone extract demonstrated the strongest free radical scavenging activity with the lowest IC_{50} value (33.9 ± 4.8 µg/mL), followed by the methanol extract (51.0 ± 1.6 µg/mL) and the aqueous extract (98.3 ± 0.4 µg/mL).Similarly, in the hydrogen peroxide scavenging assay, the aqueous extract exhibited the lowest IC_{50} value (110 ± 14.1 mg/mL), indicating greater H_2O_2 scavenging efficiency compared with the acetone (516.7 ± 5.8 mg/mL) and methanol (735 ± 49.5 mg/mL) extracts.The nitric oxide scavenging assay revealed that the methanolic extract possessed the highest NO radical scavenging activity, as evidenced by its lowest IC_{50} value (12 ± 4.2 µg/mL). The acetone and aqueous extracts showed comparatively weaker activity with IC_{50} values of 44 ± 5.7 µg/mL and 81 ± 1.4 µg/mL, respectively. Overall, the results suggest that the antioxidant potential of *Corallocarpus epigaeus* rhizome extracts is strongly influenced by the extraction solvent, with methanol favouring phenolic extraction and nitric oxide scavenging activity, whereas acetone exhibited superior flavonoid content and DPPH radical scavenging capacity.

Table 1: Extraction yield, phytochemical contents and antioxidant activities of *Corallocarpus epigaeus* rhizome extracts

Extract	Extraction Yield (% w/w)	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH IC_{50} (µg/mL)	H_2O_2 IC_{50} (mg/mL)	NO IC_{50} (µg/ML)
Methanol	6.96 ± 0.68	282.1 ±11.2	1.8 ± 0.1	51.0 ± 1.6	735.0±49.5	12.0±4.2
Acetone	5.84 ± 0.33	256.3 ±12.5	7.1 ± 0.9	33.9 ± 4.8	516.7 ± 5.8	44.0±5.7
Water	13.07 ±0.24	16.9 ± 0.4	0.1 ±0.05	98.3 ± 0.4	110.0±14.1	81.0±1.4

Values are expressed as mean ± SD (n = 3). TPC: Total Phenolic Content; TFC: Total Flavonoid Content; GAE: Gallic Acid Equivalents; QE: Quercetin Equivalents; IC_{50}: Concentration required to inhibit 50% of free radicals.

Identification and Characterization of Chemical Constituents in *Corallocarpus epigaeus* Rhizome Extract

The chemical composition of the methanol extract of *Corallocarpus epigaeus* rhizome was investigated by GC–MS analysis, and the resulting chromatogram is presented in Figure 1. A total of thirteen major compounds were identified based on their retention times and mass spectral matching with reference databases. The identified compounds, their chemical classifications, molecular formulas, molecular weights, and relative peak area percentages are summarized in Table 2.

The GC–MS profile revealed that sesquiterpenes constituted the predominant class of phytochemicals in the methanol extract. Among the identified constituents, germacrene-D was the most abundant compound, accounting for 22.01% of the total extract composition. Other major sesquiterpenes included trans-caryophyllene (12.12%), bicyclogermacrene (2.80%), α-copaene (2.12%), humulene (1.15%), γ-muurolene (0.73%), β-bourbonene (0.71%), α-amorphene (0.60%), d-elemene (0.46%), and bicycloelemene (1.28%).

In addition to sesquiterpenes, the extract contained notable quantities of palmitone (16.92%), a long-chain ketone, which represented the second most abundant compound detected in the chromatogram. Other important constituents included phytol (2.22%), a diterpene alcohol known for its antioxidant and antimicrobial properties, and squalene (1.30%), a biologically active triterpene recognized for its

antioxidant, chemopreventive, and pharmacological activities.

The predominance of sesquiterpenes and terpenoid derivatives in the methanol extract suggests that these compounds may contribute significantly to the antioxidant, antibacterial, and anticancer activities observed in the present investigation. Previous studies have reported that compounds such as germacrene-D, trans-caryophyllene, phytol, and squalene possess diverse biological activities, including free radical scavenging, antimicrobial, anti-inflammatory, and cytotoxic effects. Therefore, the biological properties exhibited by *C. epigaeus* rhizome extracts may be attributed, at least in part, to the synergistic action of these phytochemical constituents.

The GC–MS findings demonstrate that *Corallocarpus epigaeus* rhizomes represent a valuable source of bioactive terpenoids and related secondary metabolites with potential pharmaceutical significance.

Table 2: Major phytochemical compounds identified in the methanol extract of *Corallocarpus epigaeus* rhizome by GC–MS analysis

No.	RT (min)	Identified Compound	Chemical Class	Molecular Formula	Molecular Weight (g/mol)	Peak Area (%)
1	14.60	Bicycloelemene	Sesquiterpene	$C_{15}H_{24}$	204	1.28
2	14.69	D-Elemene	Sesquiterpene	$C_{15}H_{24}$	204	0.46
3	15.72	α-Copaene	Sesquiterpene	$C_{15}H_{24}$	204	2.12
4	15.94	β-Bourbonene	Sesquiterpene	$C_{15}H_{24}$	204	0.71
5	16.85	trans-Caryophyllene	Sesquiterpene	$C_{15}H_{24}$	204	12.12
6	17.48	α-Amorphene	Sesquiterpene	$C_{15}H_{24}$	204	0.60
7	17.65	γ-Muurolene	Sesquiterpene	$C_{15}H_{24}$	204	0.73
8	17.77	Humulene	Sesquiterpene	$C_{15}H_{24}$	204	1.15
9	18.40	Germacrene-D	Sesquiterpene	$C_{15}H_{24}$	204	22.01
10	18.76	Bicyclogermacrene	Sesquiterpene	$C_{15}H_{24}$	204	2.80
11	25.97	Phytol	Diterpene alcohol	$C_{20}H_{40}O$	296	2.22
12	42.05	Squalene	Triterpene	$C_{30}H_{50}$	410	1.30
13	48.41	Palmitone	Ketone	$C_{31}H_{62}O$	450	16.92

Abbreviations: RT, Retention Time; Mw, Molecular Weight.

DPPH Radical Scavenging Activity

The antioxidant potential of *Corallocarpus epigaeus* rhizome extracts was evaluated using the DPPH free radical scavenging assay. The ability of the extracts to neutralize DPPH radicals increased progressively with increasing extract concentration, indicating a concentration-dependent antioxidant response (Table1).

Among the tested extracts, the acetone extract exhibited the strongest DPPH radical scavenging activity throughout the concentration range evaluated. This was followed by the methanol extract, whereas the aqueous extract demonstrated comparatively lower antioxidant effectiveness. The superior activity of the acetone extract may be associated with its higher flavonoid content, which is known to contribute significantly to free radical scavenging mechanisms.

The antioxidant efficiency of the extracts was further assessed by calculating their IC_{50} values, representing the concentration required to inhibit 50% of DPPH radicals. As shown in Table 1, the acetone extract exhibited the lowest IC_{50} value (33.9 ± 4.8 µg/mL), indicating the highest antioxidant activity among the tested extracts. The methanol extract showed moderate activity with an IC_{50} value of 51.0 ± 1.6 µg/mL, while the aqueous extract displayed the weakest radical scavenging potential with an IC_{50} value of 98.3 ± 0.4 µg/mL. The results are presented in Figure 2A.

These findings suggest that the acetone and methanol extracts of *C. epigaeus* rhizomes possess substantial antioxidant capacity and may serve as promising sources of natural free radical scavengers.

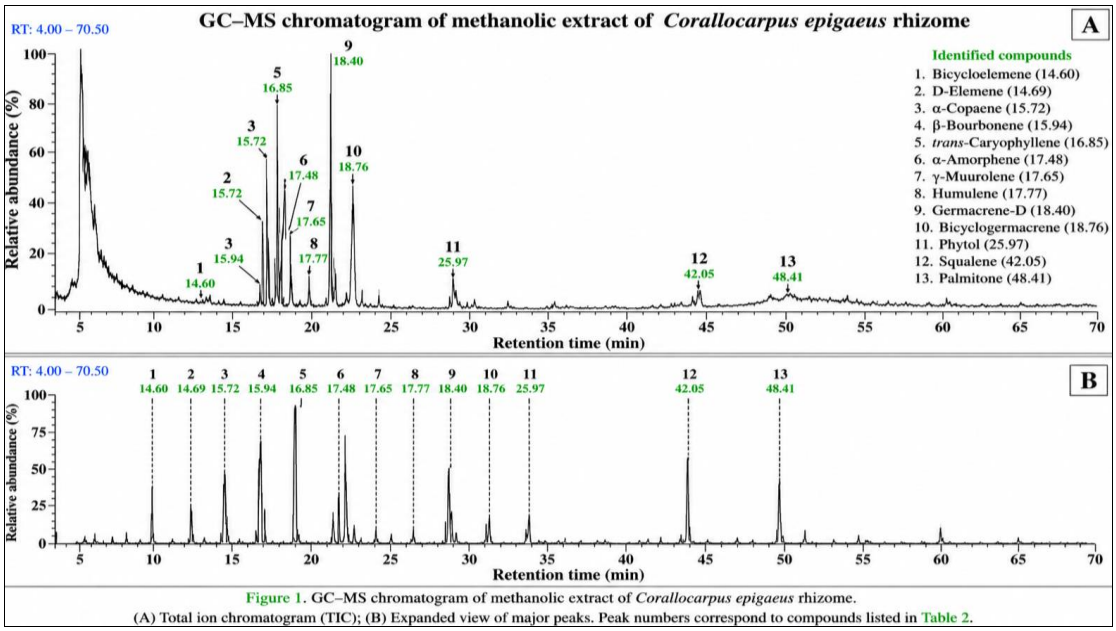

Figure 1. GC–MS chromatogram of methanolic extract of *Corallocarpus epigaeus* rhizome.
(A) Total ion chromatogram (TIC); (B) Expanded view of major peaks. Peak numbers correspond to compounds listed in Table 2.

Hydrogen Peroxide (H_2O_2) Scavenging Activity

The hydrogen peroxide scavenging potential of *Corallocarpus epigaeus* rhizome extracts was evaluated and compared with the standard antioxidant, L-ascorbic acid. The results demonstrated that all extracts possessed the ability to neutralize hydrogen peroxide radicals in a concentration-dependent manner. The results are presented in Figure 2B.

Among the tested extracts, the aqueous extract exhibited the strongest H_2O_2 scavenging activity, as indicated by its lowest IC_{50} value (110 ± 14.1 µg/mL). This activity was relatively close to that of the reference standard, L-ascorbic acid (IC_{50} = 55 ± 7.1 µg/mL). In comparison, the acetone extract showed moderate scavenging activity with an IC_{50} value of 516.7 ± 5.8 µg/mL, whereas the methanol extract displayed the weakest activity, with an IC_{50} value of 735 ± 49.5 µg/mL (Table 1).

The superior hydrogen peroxide scavenging capacity of the aqueous extract suggests the presence of water-soluble antioxidant constituents capable of effectively reducing reactive oxygen species. These findings indicate that the extraction solvent significantly influences the antioxidant behaviour of *C. epigaeus* rhizome extracts and highlights the potential of the aqueous extract as a natural source of hydrogen peroxide scavengers.

Nitric Oxide (NO) Scavenging Activity

The nitric oxide radical scavenging capacity of *Corallocarpus epigaeus* rhizome extracts was evaluated using the sodium nitroprusside–Gris reaction system. The antioxidant activity was determined by monitoring the reduction in nitrite formation, which reflects the ability of the extracts to neutralize nitric oxide radicals. The results are presented in Figure 2C.

All extracts exhibited concentration-dependent nitric oxide scavenging activity, with increasing inhibition observed at higher extract concentrations. Among the tested extracts, the methanol extract demonstrated the strongest NO radical scavenging potential, exhibiting an IC_{50} value of 12 ± 4.2 µg/mL. Notably, this activity was superior to that of the reference antioxidant, L-ascorbic acid, which showed an IC_{50} value of 16.5 ± 2.12 µg/mL.

The acetone extract displayed moderate nitric oxide scavenging activity with an IC_{50} value of 44 ± 5.7 µg/mL, whereas the aqueous extract exhibited comparatively lower activity, with an IC_{50} value of 81 ± 1.4 µg/mL (Table 1).

The remarkable nitric oxide scavenging ability of the methanol extract may be attributed to its high phenolic content, which can effectively donate electrons or hydrogen atoms to neutralize reactive nitrogen species. These findings suggest that the methanol extract of *C. epigaeus* rhizomes possesses significant antioxidant potential and may serve as a promising natural source of nitric oxide scavengers.

Reducing Power Activity

The reducing power of *Corallocarpus epigaeus* rhizome extracts was assessed by measuring their ability to convert ferric ions (Fe^{3+}) into ferrous ions (Fe^{2+}). The antioxidant potential of the extracts was compared with two standard antioxidants, L-ascorbic acid and butyrate hydroxytoluene (BHT). The results are presented in Figure 2D.

All extracts exhibited concentration-dependent reducing activity, with absorbance values increasing progressively as the extract concentration increased. This trend indicates an enhanced electron-donating capacity of the extracts at higher concentrations, reflecting their antioxidant potential.

At a concentration of 0.75 mg/mL, the aqueous extract demonstrated the highest reducing power, with an absorbance value of 0.984, followed closely by the methanol extract (0.975) and the acetone extract (0.950). Interestingly, all three extracts exhibited higher reducing activity than the reference antioxidants, L-ascorbic acid (0.920) and BHT (0.910), at the same concentration.

The observed reducing power followed the order:

Aqueous extract > Methanol extract > Acetone extract > L-ascorbic acid > BHT

The strong reducing ability of the extracts suggests the presence of bioactive compounds capable of donating electrons and terminating free radical chain reactions. These findings further support the antioxidant potential of *C. epigaeus* rhizomes and indicate that the aqueous extract possesses particularly strong reducing properties.

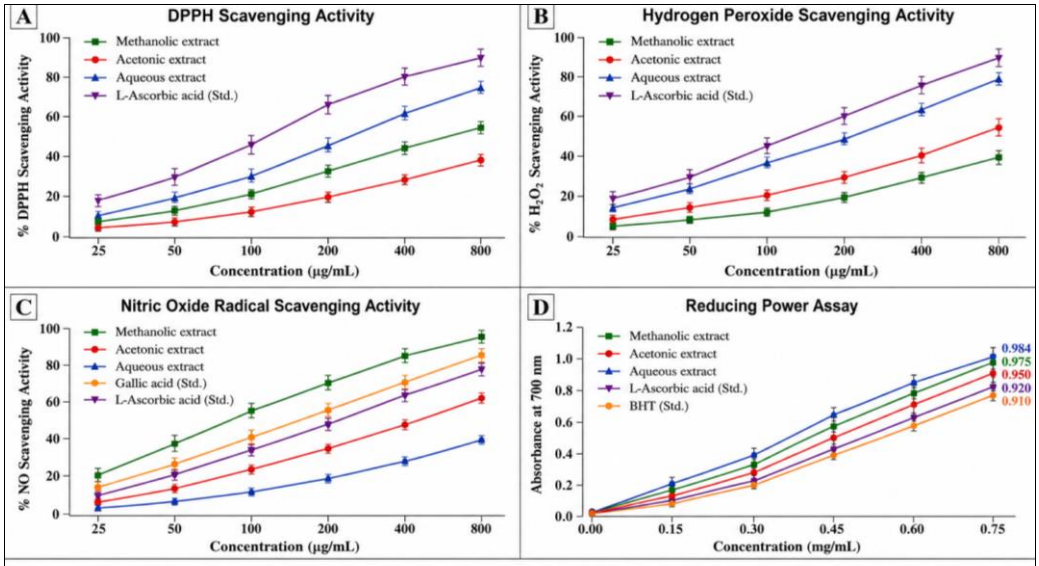

Fig. 2 The antioxidant activity leaves extracts. **a** The dose-dependent DPPH scavenging activity of leaves extracts relative to that of L-ascorbic acid. **b** The dose-dependent hydrogen peroxide scavenging activity of leaves extracts relative to that of L-ascorbic acid. **c** The dose-depended nitric oxide radical scavenging activity of leaves extracts. Gallic acid and L-ascorbic acid were used as standards. **d** The reducing power assay of different concentrations of leaves extract, L-ascorbic acid, and BHT. All results are presented as the mean ± SD (n = 3).

Anticancer Activity of *Corallocarpus epigaeus* Rhizome Extracts against Cancer Cell Lines

Effect of* Corallocarpus epigaeus *Extracts on Cell Morphology and Cell Viability:

The cytotoxic effect of *Corallocarpus epigaeus* rhizome extracts on cancer cells was initially evaluated through microscopic examination of cellular morphology following treatment with different extract concentrations. Untreated cells exhibited normal morphology, characterized by intact cell membranes, regular cell shape, and dense cellular distribution. In contrast, extract-treated cells displayed a progressive reduction in cell density accompanied by morphological alterations indicative of cellular damage, including cell shrinkage, rounding, detachment from the culture surface, and loss of intercellular contact (Figure 3).

To quantitatively assess the antiproliferative activity of the extracts, cell viability was determined using the MTT assay. As illustrated in Figure 4A, treatment with increasing concentrations of *C. epigaeus* extracts resulted in a concentration-dependent reduction in cell viability. The methanol extract exhibited the strongest cytotoxic effect among the tested extracts, producing a substantial decrease in viable cell numbers at higher concentrations.

The inhibitory effect of the methanol extract was further confirmed by the calculated IC_{50} value, indicating its pronounced antiproliferative activity against the tested cancer cell line. The observed reduction in cell viability suggests that bioactive phytochemicals present in the rhizome extract effectively suppress cellular proliferation and induce cytotoxic responses.

To verify whether growth inhibition was associated with nuclear damage and apoptosis, Hoechst 33342 nuclear staining was performed. Fluorescence microscopic observations revealed distinct apoptotic features in treated cells, including chromatin condensation, nuclear fragmentation, and the formation of apoptotic bodies (Figure 4B). These apoptotic characteristics became increasingly evident with increasing extract concentration, indicating a dose-dependent induction of programmed cell death.

Collectively, these findings demonstrate that *Corallocarpus epigaeus* rhizome extracts possess significant antiproliferative activity and are capable of inducing apoptotic cell death in cancer cells, thereby highlighting their potential as a source of natural anticancer agents.

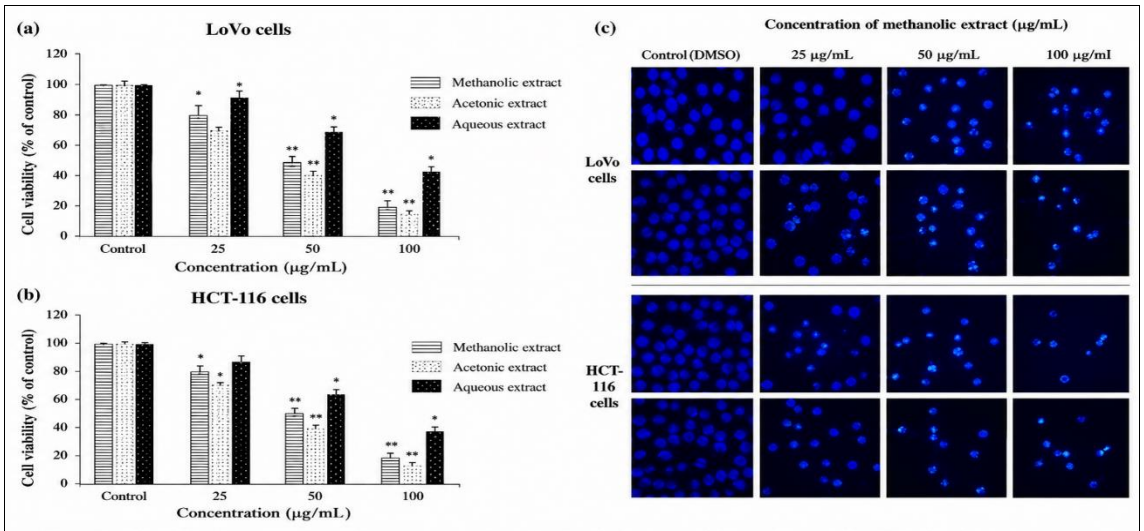

Fig. 4. The effect of *Corallocarpus epigaeus* rhizome extracts on the proliferation of colon cancer cell lines as determined by MTT and nucleus staining assays. Cell viability of (a) LoVo cells and (b) HCT-116 cells measured by MTT assay (n = 5). (c) Representative photomicrographs of nuclei stained with Hoechst 33342 after treatment with methanolic extract of *C. epigaeus* rhizomes at the indicated concentrations for 24 h. Control cells were treated with DMSO. Data are expressed as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.005$ vs. control.

Cytotoxic Effect of *Corallocarpus epigaeus* Rhizome Extracts

The cytotoxic potential of *Corallocarpus epigaeus* rhizome extracts was further evaluated by measuring lactate dehydrogenase (LDH) release, a biochemical marker of cell membrane damage and loss of cellular integrity. The results demonstrated that treatment with the extracts resulted in a concentration-dependent increase in LDH leakage, indicating progressive cellular damage and cytotoxicity (Figure 5).

In all tested groups, LDH release increased significantly with increasing extract concentrations, suggesting that the rhizome extracts effectively compromised membrane integrity and promoted cell death. Among the different extracts, the methanol extract exhibited the strongest cytotoxic effect, followed by the acetone and aqueous extracts.

At the highest concentration tested, the extracts produced substantial cytotoxicity, as evidenced by elevated LDH levels in the culture medium. The observed increase in LDH release was consistent with the reduction in cell viability detected in the MTT assay and further supported the antiproliferative activity of the extracts.

The enhanced cytotoxic response observed at higher concentrations indicates that the bioactive phytochemicals present in *C. epigaeus* rhizomes may induce membrane disruption and cellular degeneration, ultimately leading to cell death. These findings corroborate the morphological alterations and apoptotic changes observed in treated cells and confirm the significant anticancer potential of the rhizome extracts.

Overall, the LDH assay demonstrated that *Corallocarpus epigaeus* extracts exert pronounced cytotoxic effects on cancer cells in a dose-dependent manner, thereby supporting their potential application as natural anticancer agents.

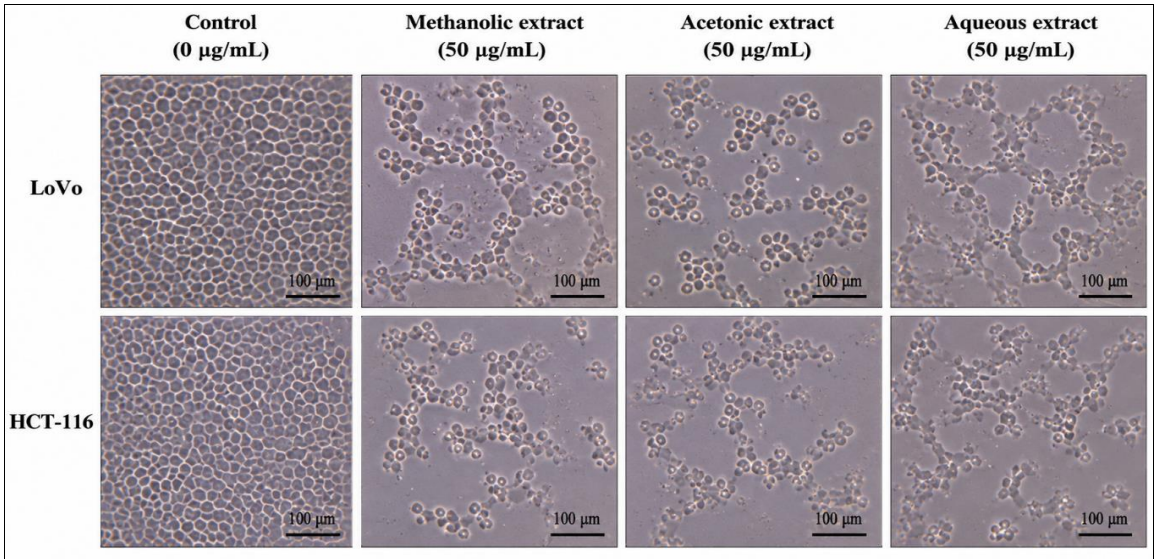

Figure 3. Representative photomicrographs showing morphological changes in LoVo and HCT-116 colon cancer cells following treatment with methanolic, acetonic, and aqueous extracts of *Corallocarpus epigaeus* rhizomes (50 µg/mL) for 24 h. Cells were cultured in DMEM supplemented with 10% FBS. Scale bar = 100 µm.

Effect of *Corallocarpus epigaeus* Rhizome Extracts on Cancer Cell Migration:

The influence of *Corallocarpus epigaeus* rhizome extracts on the migratory behaviour of cancer cells was evaluated using an *in vitro* wound healing assay. Cell migration is a critical process involved in cancer invasion and metastasis; therefore, inhibition of migration is considered an important indicator of anticancer potential. Microscopic examination revealed that untreated control cells rapidly migrated into the scratched area, resulting in substantial wound closure within 24 h. In contrast, cells treated with *C. epigaeus* extracts exhibited a marked reduction in migratory activity, as evidenced by the persistence of wider cell-free regions throughout the incubation period (Figure 6).

The methanol and acetone extracts showed the strongest inhibitory effects on cell migration, significantly reducing wound closure compared with the control group. The extent of migration inhibition increased with exposure time, indicating that the extracts effectively suppressed the movement of cancer cells into the wounded area. The aqueous extract also exhibited inhibitory activity, although its effect was comparatively weaker than that of the organic solvent extracts. Quantitative analysis demonstrated that treated cells displayed significantly lower wound closure percentages than untreated cells after 24 h of incubation ($P < 0.005$). The reduction in migratory capacity suggests that bioactive constituents present in the rhizome extracts interfere with cellular mechanisms involved in motility and migration.

The observed anti-migratory activity, together with the cytotoxic and antiproliferative effects demonstrated in the MTT and LDH assays, highlights the potential of *Corallocarpus epigaeus* rhizome extracts as promising natural agents for the suppression of cancer progression and metastasis.

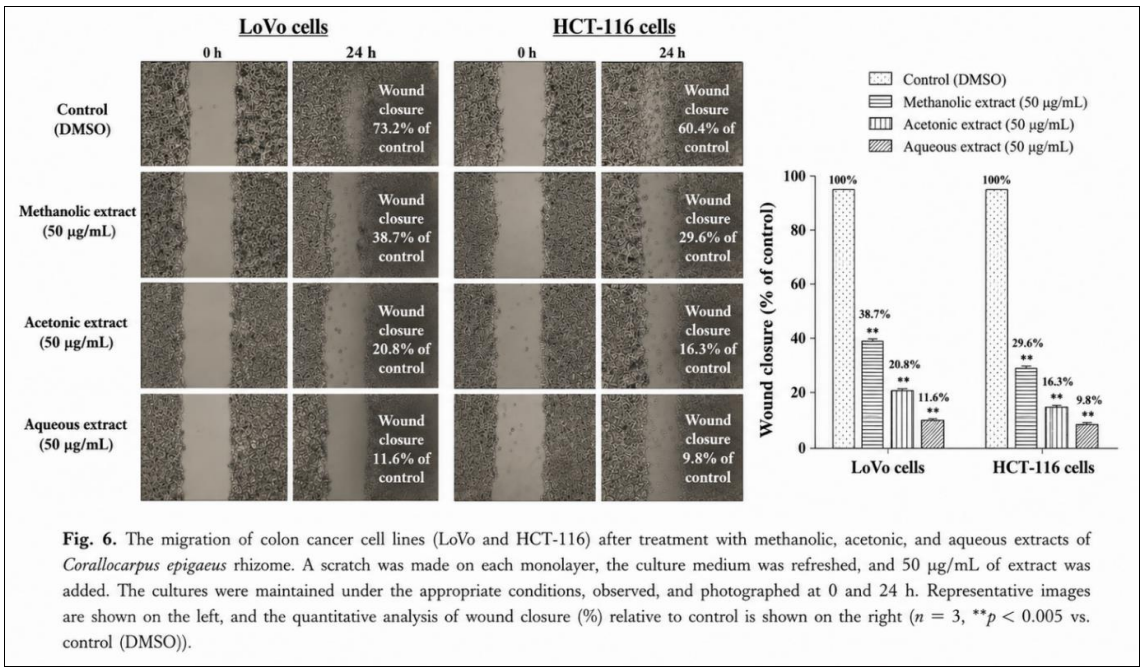

Fig. 6. The migration of colon cancer cell lines (LoVo and HCT-116) after treatment with methanolic, acetonic, and aqueous extracts of *Corallocarpus epigaeus* rhizome. A scratch was made on each monolayer, the culture medium was refreshed, and 50 µg/mL of extract was added. The cultures were maintained under the appropriate conditions, observed, and photographed at 0 and 24 h. Representative images are shown on the left, and the quantitative analysis of wound closure (%) relative to control is shown on the right ($n = 3$, $^{**}p < 0.005$ vs. control (DMSO)).

Antibacterial Activity of *Corallocarpus epigaeus* Rhizome Extracts: The antibacterial efficacy of methanol, acetone, and aqueous extracts of *Corallocarpus epigaeus* rhizomes was evaluated against selected Gram-positive and Gram-negative bacterial pathogens using the agar well diffusion method. Antibacterial activity was assessed by measuring the diameter of the inhibition zones formed around the wells after 24 h of incubation, and the results are summarized in Table 3. Among the tested extracts, the methanol extract exhibited the broadest spectrum of antibacterial activity, inhibiting the growth of all tested bacterial strains. Notably, the methanol extract demonstrated pronounced activity against *Staphylococcus aurous*, producing a larger inhibition zone than the standard antibiotic tetracycline. The strong antibacterial effect of the methanol extract may be attributed to its high phenolic content and the presence of biologically active terpenoid compounds identified by GC–MS analysis.

The acetone extracts also displayed considerable antibacterial activity against most of the tested microorganisms, including both Gram-positive and Gram-negative bacteria. However, its inhibitory effect against *S. aurous* was comparatively weaker. Among the Gram-negative organisms, *Pseudomonas aeruginosa* exhibited notable sensitivity to the acetone extract, which produced a larger inhibition zone than that observed for the standard antibiotic control. The aqueous extract showed comparatively lower antibacterial activity than the organic solvent extracts. Nevertheless, moderate inhibition was observed against certain bacterial strains, particularly *Enterococcus faecalis* and selected Gram-negative organisms. The reduced activity of the aqueous extract may be related to its lower concentration of flavonoids and other antimicrobial phytochemicals.

Overall, the antibacterial activity of the extracts varied depending on the extraction solvent and bacterial species tested. The superior performance of the methanol and acetone extracts suggests that these solvents were more effective in extracting antimicrobial constituents from *C. epigaeus* rhizomes. The results indicate that *Corallocarpus epigaeus* possesses significant antibacterial potential and may serve as a promising natural source of bioactive compounds for the development of antimicrobial agents.

Table 3: Antibacterial activity of methanol, acetone, and aqueous extracts of *Corallocarpus epigaeus* rhizomes against selected bacterial strains. Data are expressed as mean inhibition zone (mm) ± SD (n = 3). DMSO and tetracycline (30 µg) were used as negative and positive controls, respectively

Extract / Control	Gram-Positive Bacteria			Gram-Negative Bacteria			
	B. subtilis	S. aureus	E. faecalis	E. coli	P. aeruginosa	K. pneumoniae	S. typhimurium (LT2)
Acetone extract	11.5 ± 0.5	ND	8.0 ± 0.0	12.6 ±0.6	24.6 ±0.6	11.7 ±0.6	11.5 ± 0.5
Methanol extract	15.6 ± 0.6	16.5 ± 0.5	7.9 ± 0.1	15.5 ±0.5	18.3 ±0.6	11.7 ±0.6	12.3 ± 1.5
Aqueous extract	ND	ND	9.5 ± 0.5	11.6 ±0.5	17.0 ±1.0	10.5 ±0.5	12.2 ± 0.3
DMSO (Negative control)	ND	ND	ND	ND	ND	ND	ND
Tetracycline (30 µg)	16.2 ± 0.8	14.8 ± 0.4	12.7 ± 0.4	19.3 ±1.5	18.7 ±0.6	18.5 ±0.4	14.3 ± 0.3

ND = No detectable inhibition zone

Discussion

Medicinal plants have long served as valuable sources of therapeutic agents and continue to play an important role in modern drug discovery. Traditional medicinal systems have utilized numerous plant species for the treatment of various ailments, including infectious diseases, inflammatory disorders, and cancer. Among these medicinal plants, *Corallocarpus epigaeus* has attracted increasing scientific interest due to its ethno medicinal importance and the presence of diverse bioactive constituents. However, comprehensive studies investigating its phytochemical composition and biological activities remain limited. Therefore, the present study aimed to evaluate the phytochemical profile, antioxidant potential, anticancer activity, and antibacterial properties of *C. epigaeus* rhizome extracts prepared using solvents of different polarities.

One of the major challenges in phytochemical research is the complexity of plant extracts, as a single plant may contain hundreds of secondary metabolites with distinct biological functions. The efficiency of extracting these compounds largely depends on the solvent system employed. In the present study, methanol, acetone, and water were selected as extraction solvents because of their differing polarities and extraction capabilities. The aqueous extract produced the highest extraction yield, suggesting the abundance of water-soluble constituents within the rhizome. However, the methanol and acetone extracts exhibited higher concentrations of phenolic and flavonoid compounds, indicating that organic solvents were more effective in extracting biologically active secondary metabolites.

GC–MS analysis of the methanol extract identified thirteen major phytochemical constituents, including germacrene-D (22.01%), palmitone (16.92%), trans-caryophyllene (12.12%), bicyclogermacrene (2.80%), phytol (2.22%), α-copaene (2.12%), humulene (1.15%), and squalene (1.30%). These compounds have previously been associated with a wide range of pharmacological activities. Germacrene-D, the predominant constituent, has been reported to possess antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. Similarly, trans-caryophyllene is known for its anti-inflammatory, antibacterial, and cytoprotective activities. Phytol has been shown to induce apoptosis and inhibit tumour cell proliferation, while squalling exhibits antioxidant and chemo preventive effects. The presence of these compounds may collectively contribute to the biological activities observed in the present investigation.

Phenolic and flavonoid compounds are recognized as important natural antioxidants because of their ability to donate electrons or hydrogen atoms and neutralize reactive oxygen species. The methanol extract exhibited the highest total phenolic content, whereas the acetone extract showed the highest flavonoid concentration. These findings are consistent with the antioxidant assays, where both extracts demonstrated strong free radical scavenging activities. The superior DPPH radical scavenging activity of the acetone extract may be attributed to its elevated flavonoid content,

whereas the remarkable nitric oxide scavenging activity of the methanol extract may be associated with its higher concentration of phenolic compounds.

Oxidative stress plays a critical role in the initiation and progression of several chronic diseases, including cancer. Therefore, compounds capable of scavenging free radicals may provide protection against oxidative damage. In the present study, all extracts exhibited concentration-dependent antioxidant activity in DPPH, hydrogen peroxide, nitric oxide scavenging, and reducing power assays. The ability of *C. epigaeus* extracts to neutralize reactive oxygen and nitrogen species suggests their potential application as natural antioxidant agents capable of reducing oxidative stress-mediated cellular damage.

The anticancer activity of *C. epigaeus* rhizome extracts was evaluated using LoVo and HCT-116 colon cancer cell lines. Morphological observations revealed a reduction in cell density and noticeable alterations in cellular architecture following treatment. The MTT assay demonstrated significant inhibition of cell proliferation in a concentration-dependent manner, indicating strong antiproliferative activity. These findings were further supported by LDH release analysis, which demonstrated increased membrane damage and cytotoxicity in treated cells.

Hoechst 33342 staining revealed nuclear condensation, chromatin fragmentation, and apoptotic body formation in treated cells, indicating that the extracts induced apoptosis-mediated cell death. Apoptosis is considered one of the most desirable mechanisms for eliminating malignant cells because it prevents uncontrolled proliferation without causing excessive inflammatory responses. Therefore, the ability of *C. epigaeus* rhizome extracts to induce apoptosis enhances their therapeutic significance as potential anticancer agents.

Cancer metastasis remains one of the leading causes of cancer-related mortality worldwide. The wound healing assay performed in this study demonstrated that methanol and acetone extract significantly inhibited the migration of LoVo and HCT-116 cells compared with untreated controls. Reduced wound closure indicates suppression of cellular motility and migration, suggesting that the extracts may interfere with pathways involved in tumour invasion and metastatic progression. Such anti-migratory effects complement the antiproliferative and cytotoxic activities observed in the present study.

The antibacterial investigation revealed that the methanol extract exhibited broad-spectrum activity against both Gram-positive and Gram-negative bacterial strains. The acetone extracts also demonstrated considerable antibacterial activity, whereas the aqueous extract showed comparatively lower effectiveness. The antimicrobial effects observed may be attributed to the presence of terpenoids, phenolic compounds, and other secondary metabolites capable of disrupting bacterial cell membranes, inhibiting essential enzymes, and interfering with microbial metabolism. These findings support the traditional use of *C. epigaeus* and highlight its potential as a natural antimicrobial agent.

Overall, the results of the present investigation demonstrate that *Corallocarpus epigaeus* rhizomes are rich in biologically active phytochemicals possessing significant antioxidant, anticancer, anti-migratory, and antibacterial activities. The synergistic interaction among phenolic compounds, flavonoids, sesquiterpenes, and other phytoconstituents identified by GC–MS analysis is likely responsible for the observed pharmacological effects. Further studies involving bioactive compound isolation, molecular mechanism analysis, *in vivo* experimentation, and clinical validation are required to fully explore the therapeutic potential of this medicinal plant.

Oxidative stress (OS) is widely recognized as a major contributing factor in the development of numerous pathological disorders, including cancer, inflammation, cardiovascular diseases, and neurodegenerative conditions. Excessive production of reactive oxygen and nitrogen species (RONS) can overwhelm endogenous antioxidant defence systems, leading to cellular damage and disease progression. Consequently, considerable attention has been directed toward identifying natural antioxidants from medicinal plants. Previous studies have demonstrated that plant-derived phenolics, flavonoids, and terpenoids possess remarkable free radical scavenging abilities, and a positive correlation between antioxidant activity and phenolic content has frequently been reported.

In the present study, the antioxidant potential of methanol, acetone, and aqueous extracts of *Corallocarpus epigaeus* rhizomes was evaluated using DPPH radical scavenging, hydrogen peroxide scavenging, nitric oxide scavenging, and reducing power assays (Figure 2). Among the tested extracts, the acetone extract exhibited the strongest DPPH radical scavenging activity, as evidenced by its lowest IC_{50} value (33.9 ± 4.8 µg/mL), followed by the methanol and aqueous extracts. The superior activity of the acetone extract may be attributed to its higher flavonoid content, which can effectively donate hydrogen atoms to neutralize free radicals.

Interestingly, the aqueous extract demonstrated the highest hydrogen peroxide scavenging activity, with an IC_{50} value of 110 ± 14.1 µg/mL, indicating its effectiveness in eliminating peroxide radicals. In contrast, the methanol extract showed the strongest nitric oxide scavenging activity (IC_{50} = 12 ± 4.2 µg/mL), surpassing the other extracts and suggesting the presence of potent phenolic antioxidants capable of inhibiting reactive nitrogen species. These findings indicate that different classes of phytochemicals extracted by different solvents contribute to distinct antioxidant mechanisms.

The reducing power assay further confirmed the antioxidant capacity of *C. epigaeus* rhizome extracts. All extracts exhibited concentration-dependent reducing activity, reflecting their electron-donating ability and potential to terminate free radical chain reactions. At a concentration of 0.75 mg/mL, the aqueous extract displayed the highest reducing power, followed by the methanol and acetone extracts. Notably, all three extracts demonstrated reducing activities comparable to or greater than those of the standard antioxidants, L-ascorbic acid and butyrate hydroxytoluene (BHT). The strong reducing power observed in the present study may be associated with the presence of phenolic compounds, flavonoids, and terpenoid constituents identified through GC–MS analysis.

The remarkable antioxidant activities observed for *Corallocarpus epigaeus* rhizome extracts are likely attributable to the synergistic effects of phenolic compounds and bioactive terpenoids such as germacrene-D, trans-caryophyllene, phytol, and squalene. These compounds have previously been reported to possess significant antioxidant properties and may contribute collectively to the scavenging

of reactive oxygen species and the prevention of oxidative cellular damage. Therefore, the antioxidant potential of *C. epigaeus* rhizomes supports their possible application as a natural source of therapeutic antioxidants for the management of oxidative stress-related diseases.

In recent years, increasing attention has been directed toward the discovery of plant-derived compounds with anticancer properties because of the limitations associated with conventional cancer therapies, including toxicity, adverse side effects, and the development of drug resistance. Natural products have emerged as promising candidates due to their ability to target multiple signalling pathways involved in tumour initiation, progression, and metastasis. Despite the traditional medicinal importance of *Corallocarpus epigaeus*, scientific investigations exploring its anticancer potential remain limited. Therefore, the present study evaluated the antiproliferative, cytotoxic, and anti-migratory activities of *C. epigaeus* rhizome extracts against LoVo and HCT-116 human colon cancer cell lines.

The results demonstrated that methanol, acetone, and aqueous extracts of *C. epigaeus* rhizomes significantly reduced the viability of both colon cancer cell lines in a concentration-dependent manner. Morphological examination revealed noticeable alterations in treated cells, including reduced cell density, cell shrinkage, rounding, and loss of cellular integrity, indicating the induction of cellular damage. The antiproliferative activity observed in the MTT assay was further supported by LDH release analysis, which showed increased membrane damage and cytotoxicity with increasing extract concentrations.

The cytotoxic effects of the extracts were further confirmed by Hoechst 33342 nuclear staining. Treated cells exhibited characteristic apoptotic features, including chromatin condensation, nuclear fragmentation, and apoptotic body formation. These observations suggest that the rhizome extracts induce programmed cell death through apoptosis rather than nonspecific necrosis. Although the precise molecular mechanism was not investigated in the present study, previous reports have indicated that phytochemicals from medicinal plants may regulate apoptosis through activation of capsizes, induction of mitochondrial dysfunction, cell cycle arrest, and modulation of signalling pathways such as MAPK, p53, PI3K/Akt, and EGFR. Therefore, similar mechanisms may contribute to the anticancer effects observed for *C. epigaeus* extracts.

Metastasis is one of the most critical factors contributing to cancer-related mortality, and inhibition of cancer cell migration is considered an important therapeutic strategy. The wound healing assay performed in this study demonstrated that methanol and acetone extract significantly suppressed the migratory capacity of LoVo and HCT-116 cells. Treated cells exhibited reduced wound closure compared with untreated controls, indicating impaired cell motility. These findings suggest that the bioactive constituents present in *C. epigaeus* rhizomes may interfere with molecular processes involved in migration, invasion, and metastatic progression.

The remarkable anticancer activity observed in this investigation may be attributed to the phytochemical constituents identified through GC–MS analysis. Germacrene-D, the major compound detected in the methanol extract, has been reported to possess cytotoxic, antioxidant, and anticancer properties against various tumour cell lines. In addition, trans-caryophyllene, humulene, phytol, bicyclogermacrene, and squalene have been associated with apoptosis induction, growth inhibition, anti-inflammatory activity, and suppression of tumour progression. The synergistic interaction among these bioactive compounds may account for the significant antiproliferative and anti-migratory effects observed against colon cancer cells.

Collectively, the present findings demonstrate that *Corallocarpus epigaeus* rhizome extracts possess significant anticancer potential through the inhibition of cell proliferation, induction of apoptosis, enhancement of cytotoxicity, and suppression of cell migration. These results highlight the potential value of *C. epigaeus* as a source of novel bioactive compounds for the development of complementary therapeutic strategies against colon cancer. Further studies involving molecular pathway analysis, bioactive compound isolation, and *in vivo* validation are warranted to establish its clinical relevance.

The emergence of antibiotic-resistant microorganisms has become a major global health concern, creating an urgent need for the discovery of novel antimicrobial agents from natural sources. Medicinal plants have long been recognized as valuable reservoirs of bioactive compounds capable of inhibiting the growth of pathogenic microorganisms. In the present study, methanol, acetone, and aqueous extracts of *Corallocarpus epigaeus* rhizomes exhibited varying degrees of antibacterial activity against both Gram-positive and Gram-negative bacterial strains (Table 3). The results demonstrated that the antibacterial efficacy depended on both the extraction solvent and the bacterial species tested.

Among the tested extracts, the methanol extract displayed the broadest spectrum of antibacterial activity, inhibiting all examined bacterial strains. Notably, the methanol extract exhibited remarkable activity against *Staphylococcus aurous*, producing a larger inhibition zone than the standard antibiotic tetracycline. This finding is particularly significant because *S. aurous* is one of the most common human pathogens and is frequently associated with multidrug resistance, making treatment increasingly difficult. The strong inhibitory activity observed against this organism suggests that *C. epigaeus* rhizomes may contain compounds capable of overcoming bacterial defence mechanisms.

The acetone extracts also demonstrated considerable antibacterial activity and was particularly effective against *Pseudomonas aeruginosa*, producing an inhibition zone larger than that observed for tetracycline. Since *P. aeruginosa* is an opportunistic pathogen known for its intrinsic resistance to many antibiotics, this result highlights the potential therapeutic importance of the bioactive constituents present in the rhizome extracts. The aqueous extract showed comparatively lower antibacterial activity, although moderate inhibition was observed against several bacterial strains, including *Enterococcus faecalis* and selected Gram-negative bacteria.

The antibacterial properties observed in the present study may be attributed to the phytochemical constituents identified by GC–MS analysis. Several of the detected compounds, including germacrene-D, trans-caryophyllene, bicyclogermacrene, humulene, phytol, α-copaene, and palmitone, have previously been reported to possess antimicrobial activity. These compounds may exert their effects through disruption of bacterial cell membranes, interference with enzymatic systems, inhibition of protein

synthesis, and alteration of cellular metabolism. Furthermore, the high phenolic content observed in the methanol extract may contribute to bacterial growth inhibition through oxidative damage and membrane destabilization.

The superior antibacterial activity of the methanol and acetone extracts suggests that organic solvents are more efficient in extracting antimicrobial phytochemicals from *C. epigaeus* rhizomes. The observed broad-spectrum activity against both Gram-positive and Gram-negative bacteria supports the traditional medicinal use of this plant and indicates its potential as a source of natural antimicrobial agents. Further investigations focusing on the isolation of active compounds, determination of minimum inhibitory concentrations (MICs), and elucidation of antibacterial mechanisms are necessary to validate its therapeutic applications.

Overall, the findings of the present study demonstrate that *Corallocarpus epigaeus* rhizomes possess significant antibacterial properties and may serve as a promising natural resource for the development of novel antimicrobial formulations against pathogenic and drug-resistant microorganisms.

Conclusion

The present study demonstrated that *Corallocarpus epigaeus* rhizomes are a valuable source of biologically active phytochemicals with significant antioxidant, anticancer, and antibacterial properties. GC–MS analysis of the methanol extract revealed the presence of several bioactive constituents, including germacrene-D, trans-caryophyllene, phytol, humulene, bicyclogermacrene, α-copaene, squalene, and palmitone, which may collectively contribute to the observed pharmacological activities.

Among the tested extracts, the methanol and acetone extracts exhibited remarkable free radical scavenging activity, potent antibacterial effects, and significant cytotoxicity against LoVo and HCT-116 colon cancer cell lines. Furthermore, the extracts effectively inhibited cancer cell proliferation, induced apoptotic nuclear changes, increased LDH-mediated cytotoxicity, and suppressed cell migration, indicating their potential role in preventing tumour progression and metastasis.

The findings of this study support the traditional medicinal importance of *C. epigaeus* and suggest that its rhizomes may serve as a promising natural source of therapeutic agents for the management of oxidative stress-related disorders, microbial infections, and colorectal cancer. However, further investigations involving bioactive compound isolation, molecular mechanism studies, *in vivo* experiments, and clinical evaluations are required to validate its efficacy and safety before pharmaceutical applications can be considered.

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