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Original research

Phytochemical Profiling, Antioxidant Activity, and Antimicrobial Potential of *Curcuma aromatica* Rhizomes from Central India

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ABSTRACT

Curcuma aromatica Salisb. (Zingiberaceae) is a medicinal plant native to the forests of Amarkantak, Central India. The present study aims to investigate the polyphenol content, as well as the antimicrobial and antioxidant properties of *Curcuma aromatica* Rhizomes. The qualitative analysis revealed the presence of pharmaceutically important phytochemicals such as tannins, flavonoids, phenols, quinones, terpenoids, coumarins, steroids, cardiac glycosides, phlobatannins, and alkaloids in both methanol (ME) and aqueous extracts (AE) which may contribute to their observed pharmacological properties. However, saponins were absent in the ME but present in the AE. Similarly, anthraquinones were detected only in the ME, whereas glycosides were absent in both ME and AE. Quantitative assessment of total phenols and flavonoids showed significantly higher content in ME. The in vitro antioxidant activity was assessed using total antioxidant activity (TAA), DPPH, and ABTS assays, revealing markedly higher activity in the ME. It showed significantly greater TAA (39.70 ± 0.13 mg AAE g^{-1} dw; $p < 0.0001$), strong DPPH scavenging (66.01% at 220 µg/mL), and lower IC_{50} values for DPPH and ABTS. Further, FTIR examination of ME identified functional groups such as OH, CH, C=C, N-O, C-O, and C-F, indicating the presence of distinct metabolites. The GC–MS analysis confirmed the presence of 48 volatile metabolites in the ME, with the predominant compounds being tumerone, curlone (β-turmerone), hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, (E)-atlantone, and benzene, (1,1,4,6,6-pentamethylheptyl). The antimicrobial activity of the ME was evaluated using the agar well diffusion method, wherein strong antibacterial effects against *X. oryzae* (21.33 ± 0.57 mm at 15 mg/mL), *B. cereus* (16.33 ± 0.57 mm), and *Microbacterium* sp. (12 ± 0 mm) were noted. However, only partial activity against *Alternaria alternata* and *Fusarium oxysporum* were observed, while the AE showed no activity. The rhizomes of *Curcuma aromatica* contain a variety of bioactive compounds that show notable antioxidant and antimicrobial properties. However, detailed studies focusing on the isolation and characterization of these active constituents are necessary to confirm and better understand their biological effects.

1. Introduction

Nature offers a vast reservoir of medicinally valuable resources. Since ancient times, plants have served as an important source of raw materials for both traditional and modern medicine (Hosea *et al.*, 2018). Even today, nearly one-fourth of the global population continue to rely predominantly on medicinal plants to treat various infectious diseases because of their affordability, accessibility, and minimal side effects (Palhares et al., 2015; Mbuni et al., 2020). The therapeutic efficacy of these plants is primarily attributed to the presence of specific phytochemicals with pharmacological significance (Yu et al., 2021). Notably, natural antioxidants are widely recognized for their safety and efficacy in alleviating oxidative stress and protecting cells from free radical-induced damage (Engwa et al., 2022).

The increasing ineffectiveness of synthetic and semi-synthetic antibiotics, due to rising microbial resistance and escalating healthcare costs, has prompted a search for safer and more sustainable alternatives (Muteeb et al., 2023). In this context, plant-derived compounds have gained significant attention as cost-effective antimicrobial agents (Hassine et al., 2014). Unlike plants, human cells possess limited antioxidant-producing capability, rendering them more susceptible to oxidative stress (Matkowski, 2008). Furthermore, the use of synthetic antioxidants has been linked to several adverse effects, including toxicity, carcinogenicity, and mutagenicity (Yehye et al., 2015). As a result, there has been a growing demand for safe and natural antioxidants, leading to increased interest in plant-based compounds that can neutralize free radicals for use in food, pharmaceutical, and nutraceutical applications.

Fungal and bacterial infections are among the most serious diseases affecting crop yields. Although synthetic pesticides provide quick and effective disease control, their excessive and improper use leaves toxic residues that harm non-target organisms, reduce biodiversity, and accelerate the development of resistance and disease recurrence (Vurro et al., 2010; Sundin et al., 2016). In light of these concerns, plant-based pesticides present a promising, eco-friendly alternative to synthetic formulations due to their biodegradability and lower ecological impact.

C. aromatica Salisb., commonly known as wild turmeric or Junglee Haldi, is an aromatic and medicinal plant belonging to the family Zingiberaceae. Its therapeutic efficacy is attributed to diverse bioactive phytochemical constituents that e xert notable physiological effects on the human body. Owing to its wide range of medicinal applications, the species holds considerable significance in India's growing herbal sector (Umar et al., 2020). Additionally, there are only a few reports about the antimicrobial properties of *C. aromatica* against the phytopathogens. Also, this study was conducted using rhizomes collected from Amarkantak, M.P., India (Located at high altitude of approximately 3,438 ft) and hence, it provides regional insights about the ethnopharmacological data of Central India. Recently, Gas Chromatography–Mass Spectrometry (GC-MS) analysis of *C. aromatica* rhizomes identified 157 compounds and the extract was found to be antimicrobial in nature (Anuoluwa et al., 2025). Another study has reported the potential of green extraction with antimicrobial and antioxidant properties of *C. aromatica* (Sainakham et al., 2023). Therefore, this study was undertaken to investigate the phytochemical composition, functional groups, and bioactive metabolites of its rhizome extracts through analytical techniques such as Fourier Transform Infrared Spectroscopy (FTIR) and GC–MS, along with evaluating their antioxidant and antimicrobial properties. The primary aim of this study was to elucidate the phytochemical, antioxidant, and antimicrobial potential of *C. aromatica* rhizomes and to establish their relevance as a sustainable source of natural bioactive compounds for pharmaceutical and agricultural applications.

2. Materials and Methods

2.1. Study Area and Geospatial mapping

C. aromatica is not classified as a threatened or endangered species under the International Union for Conservation of Nature (IUCN) conservation status; therefore, no specific governmental permissions were required for its collection. Nonetheless, institutional guidelines were strictly adhered to, and the necessary institutional permissions were obtained. Rhizomes of *C. aromatica* were collected from the Keonchi (Latitude 22°37′6.20″ N and Longitude 81°47′12.61″ E) in the Achanakmar–Amarkantak Biosphere Reserve (AABR) in compliance with these guidelines. The exact plant locations were identified using a Geographic Information System (GIS) based approach, wherein Global Positioning System (GPS) coordinates of the collection sites were recorded, and geographic distribution maps were generated using ArcGIS 10.3 software (Thakur et al., 2022) **(Fig.1)**.

The authenticity of the plant was verified by the taxonomy expert, Dr. Ravindra Shukla, Department of Botany, Indira Gandhi National Tribal University (IGNTU), Amarkantak, Madhya Pradesh, India. The voucher specimen was deposited with accession number IGNTU/DOB/2025/Zing/Ca/01 in the Department of Botany, IGNTU, Amarkantak, Madhya Pradesh, India.

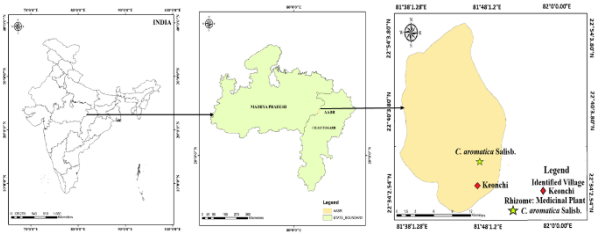


Figure 1. The map illustrates identified villages and medicinal plant collection sites (*Curcuma aromatica*) in central India, specifically within Chhattisgarh and Madhya Pradesh. The enlarged map on the right focuses on a specific study area "Keonchi" within this region.

2.2. Preparation of Plant Extracts

Fresh rhizome of *C. aromatica* were collected, washed, and dried at room temperature (RT). Thereafter, the plant material (rhizome sample) was ground in an electric blender and crushed into fine powder. Then, 10 g of powdered materials was macerated with 100 milliliters methanol and double distilled water (DDW) separately for 72 hrs. in a rotary shaker. Whatman filter papers was used to filter the macerated samples and were concentrated to dry mass in a water bath at 35-40°C. As per the experimental requirements, the collected sample residues were carefully transferred into Eppendorf tubes and stored at 4 °C to preserve their stability. For further analysis, the dried extracts were reconstituted in appropriate solvents, namely methanol and water, depending on the specific needs of the experiment.

2.3. Preliminary Qualitative Phytochemical Analysis

The phytochemical evaluation of *C. aromatica* anthocyanin, steroids, saponins, tannins, and flavonoids were carried out as per the previously described procedure (Harborne 1998). Similarly, anthraquinones, alkaloids, terpenoids, glycosides, phenols, cardiac glycosides, quinones, coumarins, phlobatannins were verified as per the protocol of Roghini and Vijayalakshmi, 2018

2.4. Quantitative Phytochemical Evaluation

2.4.1 Determination of Total Phenol Content (TPC)

The spectrophotometric approach has been used to assess the phenolics in the extract (Singleton *et al.,* 1999, Singh et al., 2021, Mansoori et al., 2025). The reaction mixture was made by combining 0.5 mL (1 mg/mL) of ME and AE, 2.5 milliliters of 10% Folin-Ciocalteu's reagent, and 7.5 % Na_2CO_3. In the blank case, 0.5 milliliters of methanol and water was used instead of extract. Absorbance was taken at λ_{max} = 750 nm using an ultraviolet-visible spectrophotometer after incubating the samples for 45 minutes at 40-45°C in the dark. A triplicate was kept to determine the sample's mean values. A calibration curve was prepared in the 20–100 µg/mL range. Finally, TPC was represented in terms of mg GAE g^{-1} of dw.

2.4.2 Determination of Total Flavonoid Content (TFC)

Total flavonoid content of the extract of rhizome was analyzed through AlCl3 colorimetric method (Chang et al., 2002; Mohan et al., 2025). Methanol and aqueous was used to dilute the rhizome extract until the concentration is 1 mg/mL, and quercetin was dissolved in methanol (20–100 µg/mL) and used to build a calibration curve. The mixture containing 0.3 milliliters diluted extracts (methanol & aqueous) and quercetin, 0.1 milliliters of 10% (w/v) $AlCl_3$, and 0.1 mL of 0.1 mM CH_3COOK has been prepared in methanol. After 25-30 minutes of incubation at 25°C, absorbance was measured at λ_{max} = 415 nm. Finally, TFC was represented in terms of mg QE g^{-1} of dw.

2.5. Antioxidant Analysis

2.5.1 Total Antioxidant Activity (TAA)

The TAA of both ME and AE were evaluated using the standard method, as described by Govindarajan et al. (2003) and Subhasree et al. (2009). In brief, the reaction mixture consisted of 0.3 ml of extract and 2.7 ml of phosphomolybdenum reagent, which contained 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. After 90 minutes of incubation at 90°C, absorbance was measured at λ_{max} = 695 nm using an ultraviolet-visible spectrophotometer. Ascorbic acid (standard curve) was prepared in the range of 20–100 µg/mL. The TAA in the extracts was expressed as mg AAE g^{-1}dw.

2.5.2 DPPH Radical Scavenging Activity Assay

Free radical scavenging activity of ME and AE was evaluated using the 2,2-diphenyl-1-picrylhydrazyl **(**DPPH) method, according to previously described methods (Kumar *et al.,* 2011, 2013; Bursal and Gulcin, 2011) (Eq. 1). A 0.004% DPPH solution in methanol was prepared to yield an absorbance of 0.98 ± 0.02 at λ_{max}=515 nm. For the assay, 2 milliliters of a solution of DPPH were mixed with 300 microliters of rhizome extract in Eppendorf tubes. Dark incubation of the samples was performed at RT for 15–20 minutes. Control was prepared likewise using 300 microliters of methanol in place of the sample. Radical scavenging activity was calculated using a standard curve generated from known concentrations (20–220 µg/mL) of the standard antioxidant. DPPH activity was calculated using the given equation:

Scavenging effect % = (Absorbance of control–Absorbance of the sample) × 100/Absorbance of control. (**Eq. 1**)

2.5.3 ABTS Radical Scavenging Assay

2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) scavenging assay is reliant on radical cation decolorization. According to this method, the reduction of $ABTS^{\bullet+}$ radicals result in the decolorization of the radical and is reliant on the plant rhizome extract's antioxidant (electron-donating) properties (Ben et al., 2017; Prasad et al., 2024). ABTS (7 mM) and potassium persulfate (2.45 mM) were dissolved in deionized water to prepare two solutions. Then, both solutions were mixed in equal proportions of 1:1 and incubated in the dark for 24 to 48 hrs. Further, $ABTS^{\bullet+}$ solution was diluted by 1:25 and aliquots of 3.0 milliliters were made in different Eppendorf tubes followed by the addition of 300 microliters of plant extract. At the end, absorbance of the solution was read at λ_{max} = 745 nm. Eq. 1 was further used to calculate the ABTS activity.

2.6. Fourier Transform Infrared Spectroscopy (FTIR) Analysis of Rhizome Extract

FT-IR analysis (Thermo Fisher Scientific, Madison, WI, USA) was performed to identify the chemical bonds and functional groups present in the ME of *C. aromatica* rhizome. The extract (1 mg/µL) was mixed with 100 mg of KBr and encapsulated into pellets. Further, sample was placed on the attenuated total reflectance (ATR) plate of the FT-IR spectrometer. The absorption spectra obtained were analyzed using BRUKER software and compared with reference data to identify and confirm the chemical bonds and functional groups present in the extract (Mansoori et al., 2022). Spectra were recorded in the range of 4000–500 cm^{-1} at a resolution of 4 cm^{-1} at RT. The ATR crystal surface was regularly cleaned with 70% ethanol during measurements (Mansoori et al., 2025).

2.7. Gas Chromatography–Mass Spectrometry (GC-MS) Analysis of Rhizome Extract

Volatile bioactive compounds in the rhizome extract were detected using GC–MS (Shimadzu GCMS-QP2010, Kyoto, Japan), equipped with a headspace sampler (AOC-20s), an auto-injector (AOC-20i), and an Rtx-5 silica capillary column. The oven temperature was initially set at 50 °C and then gradually increased to 280 °C. A uniform rhizome methanolic extract (ME) was injected into the column at a volume of 1 µL (1 mg/mL). Helium (99.99% purity) was used as the carrier gas at a flow rate of 1.2 mL/min. The mass spectrometry detector enabled the identification of individual compounds corresponding to distinct peaks. Compounds were identified based on their retention times, and quantitative estimation was carried out using the relative peak areas of the detected compounds. Finally, the spectral data obtained from individual peak components were compared with reference mass spectra from the NIST14 library for compound identification, and a complete GC–MS run was achieved within 33 minutes.

2.8. Antimicrobial Efficacy Testing

The antimicrobial efficacy of the ME and AE of *C. aromatica* was screened for its potential to serve as antibacterial and antifungal agents. The antimicrobial activity was performed against five different bacterial strains in which *Xanthomonas oryzae, Erwinia carotovora*, and *Escherichia coli* were gram-negative, whereas *Bacillus cereus and Microbacterium sp.,* were gram-positive. However, antifungal activity was tested on *Fusarium oxysporum* and *Alternaria alternata.* Agar well diffusion method was used against pathogens (bacterial and fungal), and the experiment was performed in triplicate (Prasad et al., 2024). Stock solutions of rhizome extracts were prepared using Milli-Q water for aqueous extracts and 20% methanol for methanol extracts, each at concentrations of 5 mg/mL and 15 mg/mL. Subsequently, 100 microliters of these solutions were pipetted into the prepared wells and left to diffuse for two hours. The inhibition zone diameter was measured by millimeters after incubating the plates for 24 to 72 hrs. at 30°C.

2.9. Statistical Analysis

The experiment was conducted in triplicate, with the results presented as the mean ± SD for three replicates. For the analysis of GC-MS, the data were averaged from five replicates. Statistical analysis and graphical representation were made using GraphPad Prism 8 software and OriginPro 2016.

3. Results and Discussion

3.1. Qualitative Determination of Phytochemicals

Curcuma aromatica extracts were shown to include a number of significant secondary metabolites in both ME and AE, demonstrating the plant's rich phytochemical makeup. The results are summarized in Table 1. Tannins, flavonoids, phenols, quinones, terpenoids, coumarins, steroids, cardiac glycosides, phlobatannins, and alkaloids were among the many phytochemicals found in both ME and AE; glycosides were not present in either extract. The ME was particularly effective at extracting moderately polar to non-polar chemicals because it contained anthraquinones but lacked saponins. On the other hand, the AE lacked anthraquinones but had saponins. Detection of anthraquinones in C. aromatica was not reported earlier, however, a weak positive reaction for anthraquinones was observed during the current study with ME. This may be due to trace presence of anthraquinones or methodological sensitivity differences. However, further confirmatory analyses are required to validate this finding. Overall, the phytochemical profiles of the two extracts were similar, however, there were some minor differences based on the solvent. Key bioactive substances such flavonoids, phenols, alkaloids, terpenoids, and tannins are present in *C. aromatica*, supporting its medicinal value and offering a scientific foundation for its conventional therapeutic uses. The difference in phytochemical composition between AE and ME emphasizes how solvent polarity affects extraction effectiveness.

Table 1: Preliminary phytochemical screening in methanol and aqueous extract of *C. aromatica*.

Note: Present (+); Absent (-)

Sample	Tannin	Flavonoid	Glycoside	Phenol	Quinone	Terpenoid	Coumarin	Steroid	Anthra-quinone	Cardiac glycoside	Phloba- tannin	Saponin	Alkaloid
Methanol extract	+	+	-	+	+	+	+	+	+	+	+	-	+
Aqueous extract	+	+	-	+	+	+	+	+	-	+	+	+	+

3.2. Phenol and Flavonoid Content in Rhizome Extract of *C. aromatica* Salisb.

In case of TPC, ME exhibited a higher content (53.40 ± 1.25 mg GAE g^{-1}dw) than AE (6.37 ± 0.16 mg GAE g^{-1}dw). Likewise, TFC was also higher in ME (92.03 ± 3.61 mg QE g^{-1}dw) than in AE (5.65 ± 0.09 mg QE g^{-1}dw). Results revealed that both TPC and TFC exhibited significantly ($p<0.0001$) higher levels in ME (Fig. 2). Among the most prevalent secondary metabolites in plants, phenolic compounds have a variety of biological activities, such as antioxidant, anti-inflammatory, cardioprotective, anti-aging, anticancer, and antiviral effects. One major phenolic group that greatly contributes to these therapeutic qualities is flavonoids (Han et al., 2007; Ullah et al., 2020; Sainakham et al., 2023). Comparative analysis revealed that *C. aromatica* possesses a higher phenolic content compared to other *Curcuma* species, especially *C. parviflora* and *C. latifolia*. (Burapan et al., 2020). In addition, hydroxyl groups in both phenols and flavonoids serve as a radical scavenger. In total, the ME of *C. aromatica* demonstrated significantly higher TPC and TFC, affirming its potent antioxidant potential and therapeutic value.

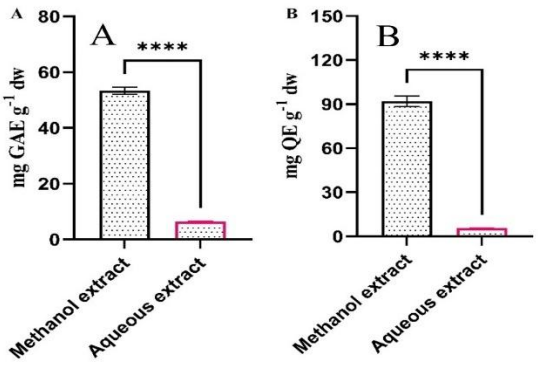


Figure 2. Total phenolic and flavonoid content of *Curcuma aromatica* extracts**.** (A) Total phenolic content (TPC) expressed as mg gallic acid equivalent (GAE) per g dry weight. (B) Total flavonoid content (TFC) expressed as mg quercetin equivalent (QE) per g dry weight. Methanol extract (ME) showed significantly higher TPC and TFC values compared to the aqueous extract (AE) (****$p < 0.0001$). Data are presented as mean ± standard deviation (n = 3).

3.3. Antioxidant and Free Radical Scavenging Activity in *C. aromatica*

Total antioxidant activity (TAA), DPPH, and ABTS free radical scavenging assays were used to assess the rhizome extract's antioxidant potential. The ascorbic acid calibration curve ($y = 0.0058x - 0.0336$, $R^2 = 0.99$) was used to determine TAA. Compared to the AE (7.25 ± 0.69 mg AAE g^{-1} dw), the ME had a substantially greater TAA (39.70 ± 0.13 mg AAE g^{-1} dw; $p < 0.0001$) (Fig. 3A)**.** ME demonstrated more radical scavenging activity than AE in the DPPH assay, attaining 66.01 ± 0.11% inhibition at 220 µg/mL, while AE only demonstrated 4.70 ± 0.29% inhibition. At the same quantity, ascorbic acid showed more action (97.55 ± 0.06%) (Fig. 3B)**.** Ascorbic acid showed somewhat higher activity at 100 µg/mL, whereas ME showed better ABTS radical scavenging activity than AE across tested doses, with inhibition surpassing that of ascorbic acid at lower concentrations (20–80 µg/mL). ABTS scavenging activity was negligible in AE (Fig. 3C). ME demonstrated considerably lower IC_{50} values for both DPPH and ABTS assays than AE ($p < 0.0001$), which is consistent with these findings. Significantly, compared to the positive control, the IC_{50} value of ME for ABTS was lower (Fig. 3D).

According to the current investigation, ME had a noticeably higher TAA potential. Strong correlations between TPC, TFC, and TAA indicate that polyphenols are key component of the rhizome extract's antioxidant activity. These compounds' capacity to donate electrons due to the presence of hydroxyl groups is probably linked to the antioxidant effects seen in DPPH and ABTS experiments (Chen et al., 2020). Plant-derived antioxidants have also been shown in earlier research to have antibacterial qualities, which could have achieved through protein regulation, substrate restriction, and inhibition of microbial enzymes (Aminov, 2010; Mansoori et al., 2020). The fact that *C. aromatica* was collected from the Amarkantak forest region, which has little anthropogenic disturbance and no use of pesticides or insecticides, may have contributed to its relatively higher TPC, TFC, TAA, and radical scavenging activities (lower IC_{50} values) (Panda et al., 2020).

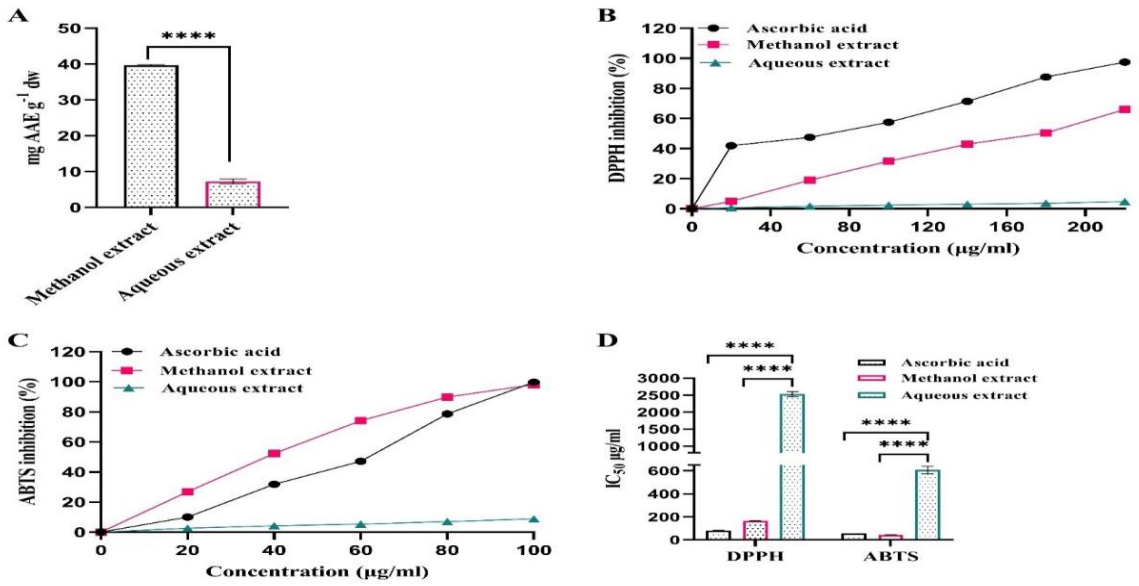


Figure 3. Antioxidant activity of methanol and aqueous extract of *C. aromatica* rhizome. (A) Total antioxidant activity (expressed as mg AAEg^{-1} dw). (B) DPPH radical scavenging activity at various different. (C) ABTS radical scavenging activity at various concentration. (D) IC_{50} values for DPPH and ABTS assays. Data is represented as mean + SD (n=3). Asterisks (****) indicates $p < 0.0001$.

3.4. Detection of Functional Group in *C. aromatica*

FTIR spectroscopy is a popular analytical method for determining the functional groups associates to pharmacologically active compounds. In the present study, functional groups found in the ME of *C. aromatica* rhizome were identified using this technique. Easmin et al. (2017) claim that this technique produces a distinctive qualitative profile and an overall chemical fingerprint of the extract, which helps in authentication and validation of GC-MS data and phytochemical screening findings. A resolution of 500–4,000 cm^{-1} was used to analyze the absorbance bands in the area (Fig. 4). Importantly, the broad absorption peak at 3253.07 cm^{-1} indicates the presence of O–H stretching vibrations, while the peak at 2914.09 cm^{-1} corresponds to C–H stretching. The absorption band at 1604.92 cm^{-1} is attributed to C=C stretching, and the peak at 1509.71 cm^{-1} represents N–O stretching. Further, the bands observed at 1275.78 cm^{-1} and 1003.28 cm^{-1} are assigned to C–O and C–F stretching vibrations, respectively. These peaks collectively suggest the presence of functional groups such as alcohols, alkanes, aromatic esters, and fluorinated compounds. **(**Supplementary Table 1**)**. The polymeric hydroxyl group is stretched in the extract spectra, as evidenced by the broad peak of OH at 3253.07 cm^{-1}, a characteristic of polyphenolic compounds (Wongsa et al., 2022). O–H, C–H, and C–O functional groups have been previously reported in plants of the Zingiberaceae family (Sohrab et al., 2024); however, the aromatic C=C stretching band at 1604.92 cm^{-1}, N–O stretching band at 1509.71 cm^{-1}, and C–F stretching band at 1003.28 cm^{-1} indicating the presence of aromatic rings, nitro or nitroso functional groups, and fluoro-containing compounds, respectively were unique to the *C. aromatica* rhizome. Similarly, O–H (hydroxyl) stretching and aromatic C=C stretching was also reported in *C. longa* rhizome extract (Arifah et al. 2026), however, aliphatic C–H stretching (2914.09 cm^{-1}), N–O stretching (1509.71 cm^{-1}), C–O stretching (1275.78 cm^{-1}), and C–F stretching (1003.28 cm^{-1}) attributed to alkane or alkyl chain components, esters, and fluoro-containing compounds respectively. Thus, FTIR analysis indicated the presence of various functional groups, suggesting a diverse range of metabolites presence in the rhizome extract. However, as FTIR analysis provides only functional group information and cannot identify specific compounds, GC–MS analysis was subsequently performed to accurately characterize the individual molecules present in the extract.

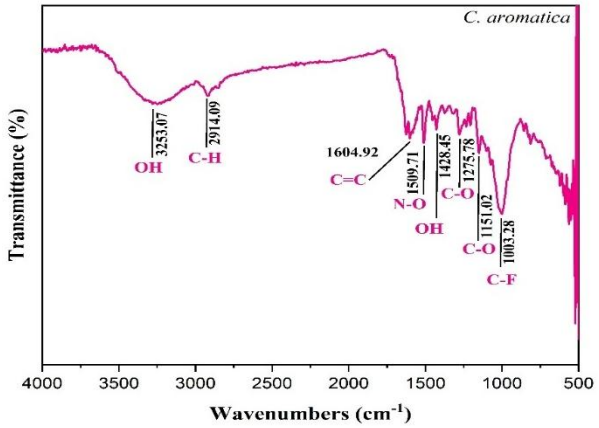


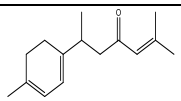
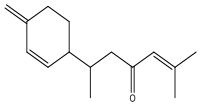
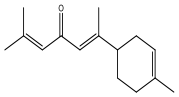
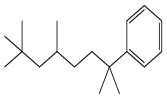
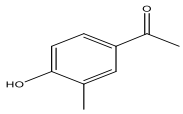
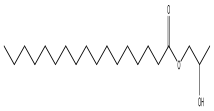
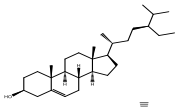
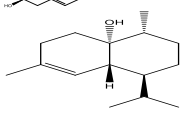
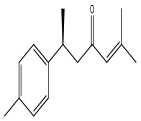
Figure 4. FT-IR spectrum of rhizome extract of *C. aromatica* and their probable functional groups with wavenumbers (4000-500 cm^{-1}).

3.5. GC-MS Analysis

In order to identify the specific volatile molecules in the extract, ME of the rhizome was subjected to GC-MS, one of the most popular methods for separating phytoconstituents along with their molecular mass. Forty-eight compounds were identified based on their retention time (RT) and peak area **(**Supplementary Table 2**)**. Major compounds were tumerone (21.64), curlone (β-turmerone) (9.19), hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (3.26), (E)-Atlantone (2.5), and Benzene, (1,1,4,6,6-pentamethylheptyl)- (1.95) (Table 2). Other compounds were also reported with lower peak area. Turmerone and curlone are the principal sesquiterpenes of Curcuma sp., which show versatility in their roles as antiparasitic (Le et al., 2019), antibacterial (Negi et al., 1999), neuroprotective (Dohare et al., 2008), and anticancer activity (Liju et al., 2013). In addition, hexadecanoic

acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester have been reported for their antioxidant and insecticidal activity (Tyagi and Agrawal, 2017; Anuoluwa et al., 2025). Recently GC-MS study of *C. aromatica* identified 157 compounds in which hexadecenoic acid methyl ester and 9,12-octadecadienoic acid methyl ester were also reported as major compound (Anuoluwa et al., 2025). The variations in identified number of compounds could be due to several reasons such as sensitivity of GC-MS column, environmental factor, post-harvest handling of sample, instrument calibration and sample preparation. Few studies describe gamma-sitosterol's antioxidant and hepatoprotective effects (Sirikhansaeng et al., 2017). Vitamin E, comprising tocopherols and tocotrienols, is a well-known antioxidant that can donate electrons to reduce oxidized compounds (Ungurianu et al., 2021). Caryophyllene oxide has antioxidant, antimicrobial, antitumor, and anti-inflammatory capacities (Xiang *et al.,* 2017). Interestingly, metabolites detected through GC-MS have several bioactivities, of which few have been reported as antioxidant agents that could have contributed to the overall antioxidant and free radical scavenging abilities of *C. aromatica* rhizome extract.

Table 2. Phytochemical compounds of methanol extract of rhizome of *C. aromatica* showing the identified compounds with retention time, peak area, molecular formula, molecular weight (M.W), mass-to-charge ratio (m/z) and chemical structure of the compound.

	Name of the compound	RT	Peak area (%)	Molecular formula	m/z (mass peak)	M.W..	Chemical Structure
1.	Tumerone	14.328	21.64	$C_{15}H_{22}O$	329	218	
2.	Curlone (β-turmerone)	14.945	9.19	$C_{15}H_{22}O$	309	218	
3.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	34.153	3.26	$C_{19}H_{38}O_4$	359	330	
4.	(E)-Atlantone	16.211	2.5	$C_{15}H_{22}O$	299	218	
5.	Benzene, (1,1,4,6,6-pentamethylheptyl)-	16.059	1.95	$C_{18}H_{30}$	268	246	
6.	4-Hydroxy-3-methylacetophenone	9.675	1.85	$C_9H_{10}O_2$	250	150	
7.	Octadecanoic acid, 2,3-dihydroxypropyl ester	39.329	1.3	$C21H_{42}O_4$	388	358	
8.	Gamma-Sitosterol	57.092	1.24	$C_{29}H_{50}O$	409	414	
9.	Cubenol	17.561	0.69	$C_{15}H_{26}O$	305	222	
10.	aR-Turmerone	18.962	0.25	$C_{15}H_{20}O$	323	216	

3.6. Antimicrobial activity

A comparative antimicrobial assay was conducted using ME and AE of *C. aromatica* rhizome. The extracts were evaluated for their inhibitory activity against five bacterial strains (Table 3). In the ME of *C. aromatica,* a notable zone of inhibition was observed against *X. oryzae, B. cereus,* and *Microbacterium sp.* (Fig. 5), while the AE exhibited no activity against the tested bacteria. The highest inhibition zone was observed against *X. oryzae*, measuring 15±0 mm and 21.33±0.57 mm at a dosage of 5 and 15 mg/ml, respectively. The measured inhibition zone against *B. cereus* was 15.66±0.57mm and 16.33±0.57 mm at both 5 and 15 mg/ml dosage, followed by 11±0 mm and 12±0 mm inhibition zone for *Microbacterium* sp. at a dosage of 5 and 15 mg/ml, respectively. ME derived from *C. aromatica* exhibited partial inhibition against *F. oxysporum* and *A. alternata* (Fig. 6), while no inhibitory activity was observed with AE.

The antimicrobial activities of extracts against *X. oryzae*, *B. cereus*, and *Microbacterium* sp., can be further explored for agricultural applications. Earlier study on *C. aromatica* has also shown the antimicrobial activities against gram negative bacteria such as such

as *E. coli, Klebsiella, Pseudomonas, Moraxella, Campylobacter* (Anuoluwa et al., 2025). These findings demonstrate that natural compounds can be targeted for plant based modern drug development. Another study elucidated an anti-inflammatory activity of *C. aromatica* rhizome extracted through green extraction method using edible deep eutectic solvents (Sainakham et al., 2023). Currently scientists are working to find out the alternatives of synthetic pesticides and antibiotics, which aligns with the increasing need for environmentally friendly agriculture practices. Targeting specific crop pathogens such as *X. oryzae*, which causes bacterial leaf blight in rice (Kumar and Ghazi 2013), *B. cereus*, which is linked to foodborne disease and spoilage, and *Microbacterium sp.*, which affects a variety of crops, demonstrates the extracts versatility in crop protection (Adhikari et al., 1995; Kulkova et al., 2023; Cordovez et al., 2018). Eventually, isolation, characterization and field testing of such antimicrobial compounds and integration in Integrated Pest Management (IPM) systems could provide a comprehensive approach to disease control, reducing dependency on chemical inputs and lowering the chance of resistance development.

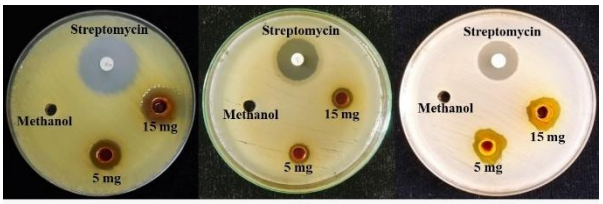


Figure 5. Antibacterial activity of methanol extract prepared from *C. aromatica* rhizome against (a) *X. oryzae,* (b) Microbacterium sp. and (c) B. cereus, showing zone of inhibition at 5 mg/ml and 15 mg/ml concentration compared to standard antibiotic streptomycin and methanol.

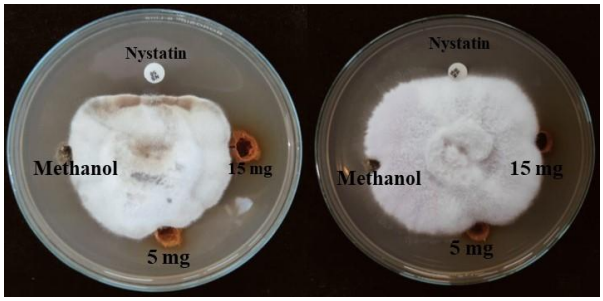


Figure 6. Antifungal activity of methanol extract of *C. aromatica* against *A. alternata* and *F. oxysporum* respectively, wherein zones of inhibition was compared with the methanol control and standard antifungal agent.

Table 3: Screening the antibacterial potential of crude extract of *C. aromatica* against bacterial infectious pathogens using the agar well diffusion assay. The zone of inhibition (mm) was shown by aqueous extract (AE), methanol extract (ME), and antibiotic streptomycin against selected pathogenic and non-pathogenic bacterial strains. The values are represented as mean ± standard deviation (mm) of triplicates.

Sample	**Zone of inhibition (mm)**					
	Concentration	*E. coli*	*X. oryzae*	*Microbacterium sp.*	*E. cartovora*	*B. cereus*
Aqueous	5 mg/ml	No Activity	No Activity	No Activity	No Activity	No Activity
Extract (AE)	15 mg/ml	No Activity	No Activity	No Activity	No Activity	No Activity
Streptomycin	10 µg/ml	29.66±0.57	33.66±0.57	24.66±0.57	30.66±0.57	21.66±0.57
Methanol	5 mg/ml	No Activity	15±0	11±0	No Activity	15.66±0.57
Extract (ME)	15 mg/ml	No Activity	21.33±0.57	12±0	No Activity	16.33±0.57
Nystatin	30 µg/ml	29.66±0.57	27.66±0.57	21.66±0.57	16.66±0.57	18.66±0.57

4. Conclusions

The present study establishes baseline information on the phytochemical profile of *Curcuma aromatica*, a species belonging to the Zingiberaceae family, thereby contributing to a better understanding of its chemical composition. The existence of various phytochemicals including tannin, flavonoid, phenol, quinone, terpenoids, coumarin, steroids, anthraquinone, cardiac glycosides, phlobatannins, saponins and alkaloids were established. The levels of antioxidant content and antimicrobial activity varied among the samples, which may be attributed to differences in the types and concentrations of phytochemicals present. These variations could, in turn, contribute to their observed ethnomedicinal significance. A positive corelation was found between phenolics and free radical scavenging activity. In order to address widespread health issues, phytochemical analysis, antioxidant and antimicrobial activity could be used to improve healthcare access and sustainable agriculture. The potential of plant-based phytochemicals can be explored as an as antimicrobial agents for both plant disease and human ailments which can provide long-term eco-friendly pest and disease management. Compounds of plant origin offer various advantages over manufactured chemical pesticides, including sustainability, low toxicity to non-target organisms, and a lower probability of resistance development. However, problems such as extraction method standardization, formulation stability, and regulatory approval processes must be addressed before they are widely adopted. Inclusively, continuing research and innovation in this field have the potential to transform agricultural practices into more environmentally friendly and sustainable alternatives while enabling the identification of novel compounds from plants and novel drug development could overcome the antimicrobial resistance. Nevertheless, further studies

are required for the identification and purification of molecules responsible for specific antioxidant and antimicrobial activities.

Declarations

Ethics approval and consent to participate

Not applicable.

Ethical consideration

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Patent Declaration: A patent was granted in the year 2024 entitled "System for Synthesizing *Curcuma aromatica* Salisb., extract and Characterizing Properties of the Extract Thereof. Patent No. 20 2024 104 723, IPC: A61 K, 36/9066

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Authors Contribution Statement

TKT and AK designed the work. DN performed the experiments and prepared the draft of the article. SND and AT corrected the article. Finally, all authors read and approved the final version of the article.

Supplementary material

The supplementary material presents the chemical profile of Curcuma aromatica rhizomes using FTIR and GC–MS analyses. Link

Data Availability Statement: All data generated or analyzed during this study are included in this published article and its supplementary information files

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