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RESEARCH ARTICLE

# Comparative phytochemical analysis, antioxidant and anti-inflammatory activities of rhizome and leaves of *Curcuma caesia* Roxb. and *Curcuma longa* L.

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## Abstract

Turmeric is a well-known medicinal herb used for traditional and medicinal purposes since ancient times. It is also an important ingredient in the cosmetic, food and pharmaceutical industries. Two common species, *Curcuma longa* L. (yellow turmeric) and *Curcuma caesia* Roxb. (black turmeric), are noted for their medicinal potential. However, commercially, yellow turmeric is more extensively explored than black turmeric. In the present study, a comparative phytochemical analysis was conducted, along with *in vitro* anti -inflammatory and antioxidant activity assessment of the rhizome and leaves of both yellow and black turmeric. GC-MS and FTIR analysis revealed a wide range of phytocompounds with known medicinal properties in both yellow and black turmeric rhizome. The presence of biologically significant phytocompounds such as methyl stearate, glycidyl oleate, curcumenol and eucalyptol in higher proportion in the rhizome of *C. caseia* compared to yellow turmeric supports further exploration of the commercial and medicinal potential of *C. caseia*. Antioxidant and anti-inflammatory analyses indicated that the rhizome of *C. caseia* possesses anti-inflammatory and antioxidant activities comparable to those of *C. longa.* This study also reports the presence of bioactive metabolites in the leaves of both black and yellow turmeric, along with anti-inflammatory and antioxidant activities in the leaves comparable to their respective rhizomes. These findings suggest that turmeric leaves could be as significant as turmeric rhizomes for medicinal and industrial applications.

## Keywords

*Curcuma longa* ; *Curcuma caesia* ; rhizome; GC-MS; FTIR; biological activity

## Introduction

*C. longa* L. commonly known as turmeric or "Haldi", is a well-known spice used in numerous food preparations for ages and is also a crucial component in many cosmetics, pharmaceuticals and food products. India is one of the major exporters of *C. longa* (1). Previous review of *C. longa* has elaborated on the herb's widespread application in treating various ailments and diseases (2). Another species, *C. caesia* Roxb. (Black turmeric), has also been reported to possess several medicinal properties, including the potential to

treat leprosy, piles, cancer, enhance fertility, heal wounds and address common ailments such as vomiting, fever, toothache etc. (3, 4). However, due to inadequate large-scale commercial cultivation (as practiced for *C. longa*) and unrestricted harvesting from natural stands, *C. caesia* has become endangered (5). Despite its numerous medicinal properties, *C. caesia* remains comparatively less explored in traditional practices as well as industrial and pharmaceutical applications (3). *C. caseia* possesses immense medicinal potential, exhibiting antimicrobial, antioxidant and analgesic activities, anticancer properties, muscle relaxant effect and more (2, 3). The medicinal potential of *C. caseia* rhizome is attributed to the presence of diverse classes of organic phenols, alkaloids, phytosterols, carbohydrates, terpenoids, saponins and other compounds. Curcumin and its derivatives are representative bioactive metabolite of turmeric species. Traditionally and industrial, applications and research interests for almost all species of turmeric have primarily focused on the rhizomes. Moreover, very few studies have been conducted on the phytochemical and medicinal properties of *C. longa* and *C. caseia.* An important objective of the present study is to explore the leaves of *C. longa* and *C. caseia* as potential source of antioxidant and anti-inflammatory compounds, which could be significance for the food, cosmetic and pharma industries. The present work was designed with the objective of conducting a comparative study of the phytocompounds and biological activities of the rhizome and leaves of methanolic extracts from *C. longa* and *C. caesia.*

## Materials and Methods

### *Reagents and standards*

The reagents and chemicals used in this study were of analytical grade. The standard drugs aspirin, diclofenac potassium and acetylsalicylic acid were used as standard for anti-inflammatory activity. BHT (Butylated Hydroxytoluene), ascorbic acid and rutin were used as standards for antioxidant activity.

### *Plant materials*

For the present study *C. caseia* and *C. longa* plants were procured from JadiButi Farm located in Kolhupani, Dehradun, Uttarakhand. The plants were maintained in the Department of Biotechnology, School of Applied and Life Sciences (SALS), Uttaranchal University, Dehradun.

### *Extract preparation*

The rhizomes of both plants were thoroughly washed to remove dust and other impurities and then dried in the shade. A 20 g sample of dried rhizome was weighed, crushed into powder form and dissolved in 100 mL of 100 % methanol. The mixture was left overnight. The sample was then placed in a Soxhlet apparatus for extraction at 40-45 ºC for 5 h (14 cycles). The solution was filtered and concentrated using a rotary evaporator to yield 2 % of dry weight of the residue. The extraction yield was calculated and expressed as a percentage of the extract in relation to the mass of crushed rhizome (%, w/w). The concentrated extract was used for analytical analysis. The extract of turmeric leaves from both species was prepared similarly, with extraction completed after 10 cycles. The percentage yield of the extract was found to be 2 % w/w. This was calculated as the yield/dry weight of the sample. 1 mg of extract and standard (selected for different biological activities) was dissolved in 10 mL methanol to obtain a concentration of 100 mg/mL. The resultant stock was diluted to obtain concentration of 25, 50 and 75 µg/mL.

### *Fourier transform infrared spectroscopy (FTIR)*

For FTIR analysis, 100 mg of KBr was finely powdered to obtain uniform consistency. The powdered KBr was used to prepare a pellet with an approximate diameter of 13 mm. Following pellet preparation, 2 drops of the methanolic extract of the rhizome or leaf of both plant species were carefully pipetted onto the pellet, which was then left at room temperature to air dry undisturbed. After the pellet was completely dried, it was placed in an FTIR analyser for analysis. The FTIR instrument model used was the Nicolet Summit LITE, with instrument serial No. BFJ2010008, an optical velocity of 0.4747, an aperture 100.0 and software OMNIC PARADIGM (6).

### *Gas chromatography-mass spectrometry (GC-MS)*

The rhizome and leaf extracts of both plant species were subjected to GC-MS analysis using previously reported methodologies (7, 8). Perkin Elmer Auto system was used as the GC-MS analyser. Phytoconstituents of the extracts were identified using the NIST 98 database. Identification of phytoconstituents was based on molecular mass, structure, retention time and mass spectra. The mass spectra of the compounds were determined using Turbo mass software.

### *DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay*

The antioxidant potential of the rhizomes and leaves of *C. longa* and *C. caseia* was determined using the standard protocol for assessing free radical scavenging activity through the DPPH assay (9). The antioxidant activity of both plant species was compared to the standard BHT (Butylated Hydroxytoluene), rutin and ascorbic acid. Stock solutions of the sample and standards were prepared at different concentrations (25 µg/mL, 50 µg/mL and 75 µg/mL). 2 mL of DPPH methanol solution (80 µg/mL) was added to each tube and incubated for 30 min in the dark at RT. Ultraviolet (UV) absorbance was recorded at 517 nm using a UV-VIS Double Beam Spectrophotometer (Systronics Au-2701). The inhibition of the free radical DPPH was calculated utilizing the formula.

$$\text{DPPH scavenging activity (\%)} = \frac{Ab\ c_{nm} - Ab\ s_{nm}}{Ab\ c_{nm}} \times 100 \quad \text{.....(Eqn. 1)}$$

Where Ab c **=** Absorption of control = 1.154 nm, Ab s **=** Absorption of sample

### *Anti-inflammatory activity*

The inhibition of albumin denaturation was studied to assess the anti-inflammatory potential of the rhizome and leaf extracts (10). Different concentrations (25 µg/mL,

50 µg/mL and 75 µg/mL) of the rhizome and leaf extracts were prepared. To each concentration of the sample, a 1 % aqueous solution of bovine albumin fraction was added and the pH of the reaction mixture was adjusted to 7.0. All samples were incubated at 37 °C for 20 min, then heated at 57 °C for 20 min. After heating, the samples were cooled and the OD (optical density) was recorded 660 nm. Independent experiments were conducted for both plant species, with each experiment repeated thrice. The anti-inflammatory potential of the rhizomes and leaves of both plant species was compared to the standard aspirin, diclofenac potassium and acetylsalicylic acid. The percentage inhibition of protein denaturation was calculated using the following formula:

Percentage inhibition (%) = $\{(Ab\ c_{nm} - Ab\ s_{nm}) \div Abc_{nm} \times 100\}$

……..(Eqn. 2)

Where Ab c= Absorption of control, Ab s= Absorption of sample

### *Statistical analysis*

The data was statistically analyzed using one-way ANOVA and expressed as mean ± SE, followed by Dunnett's test using Graphpad InStat version 7.0. Differences with $p<0.05$ were considered statistically significant.

## Results and Discussion

### *FTIR analysis*

The analysis of the FTIR spectrum of the methanolic rhizome extract of *C. caseia* (Fig. 1) revealed characteristic peaks of organic compounds from different functional groups, including hydroxyl compound at 3415 $cm^{-1}$, carbonyl compound at 2079 $cm^{-1}$, amide I and carboxylic acids at 1639 $cm^{-1}$, O-H bend, alcoholic group at 1392 $cm^{-1}$, phenolic group at 1275 $cm^{-1}$, aliphatic amines at 1019 $cm^{-1}$ and halogen compound at 590 $cm^{-1}$. Similarly, the FTIR spectrum of *C. longa* rhizome extract (Fig. 1) depicts characteristic peaks of organic compounds from diverse functional groups, including hydroxyl compounds at 3422 $cm^{-1}$, methyl group at 2949 $cm^{-1}$, cyclo alkane at 2838 $cm^{-1}$, O-H stretch, carboxylic group at 2525 $cm^{-1}$, terminal alkynes, nitrile compounds at 2176 $cm^{-1}$, ketones aldehyde at 1651 $cm^{-1}$, alkanes at 1453 $cm^{-1}$, C-O-C group at 1112 $cm^{-1}$, aliphatic amines at 1017 $cm^{-1}$ and alkene at 701 $cm^{-1}$ (11-13). A study was also reported presence of similar groups O-H, C=C, N-H,C-H, C-O) in the ethanolic extract of the rhizome of *C. caseia* (1). FTIR analysis of the leaves of *C. caseia* and *C. longa* revealed the presence of compounds belonging to similar classes as identified in the FTIR analysis of their respective rhizomes of *C. caseia* and *C. longa.* This finding indicates the presence of diverse phytocompounds in the leaves of turmeric species, similar to their rhizomes, suggesting their potential medicinal and commercial importance. The significance of the FTIR technique for spectral analysis of curcuma species has been reported in earlier studies. A study was reported the use of FTIR in association with PLS for the determination of curcumin in different *Curcuma* species (13). Similarly, a study conducted also supports the utilization of FTIR in analysing the presence of curcumin in curcuma species (14).

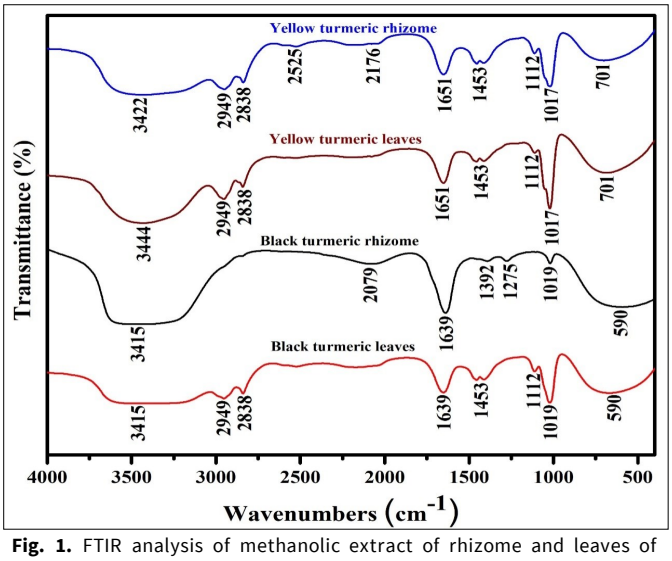


**Fig. 1.** FTIR analysis of methanolic extract of rhizome and leaves of *C. caseia* and *C. longa.*

### *GC-MS analysis*

The GC-MS technique is recognised as an effective approach for identifying various phytocompounds present in plant extracts and other plant products. Once the phytocompounds are identified, their biological significance can be researched by analysing cited literature (15), or the extract can be subjected to *in vitro* studies to evaluate the plant's medicinal and pharmacological potential. GC-MS analysis of the methanolic extract of the rhizomes of *C. longa* and *C. caseia* revealed the presence of 61 (Table 1, Fig. 2) and 72 (Table 2, Fig. 3) phytoconstituents respectively. Major compounds found in the rhizome of *C. longa* include caryophyllene, gamma-elemene, beta-elemenone, tumerone, zingiberenol, iso curcumenol, curlone, fragranyl acetate and curcumin. Other biologically active phytocompounds identified in *C. longa* include picrotoxinin, glycidyl derivatives, (E)-Atlantone, curcuphenol, germacrene B and eucalyptol. Similarly, major phytocompounds found in the rhizome of *C. caesia* include eucalyptol, camphor, isobornyl acetate, viridiflorol, epicurzerenone, curcumenol, zedernone, glycidyl derivatives and linalool oxide. Methyl stearate, glycidyl oleate, curcumenol and eucalyptol were present in higher proportions in the rhizome of *C. caesia*, while germacrene B, gamma-elemene and caryophyllene were present in higher proportions in the rhizome of *C. longa* compared to *C. caesia*.

Several phytocompounds identified in the rhizome extract have been reported to possess medicinal or pharmacological properties. For examples, 2-Bornanone has been reported to exhibit antitumor, analgesic, antibacterial, anti-inflammatory and fungicide properties (13). Gamma-elemene and curcumenol are also known for their antitumour potential (16, 17). Eucalyptol is an important bioactive compound found in several plant species, with numerous biological activities including antioxidant, anti-inflammatory and cardiovascular effect as well as roles in treating respiratory disorders, pancreatitis and colon damage (18). Tumerone is another biologically significant phytocompound in turmeric, exhibiting antibacterial, antifungal and cytotoxic activities (19). Germacrene B has been

identified with reported anti-inflammatory activity (20). Similar to the findings of the present study, researchers conducted GC-MS analysis of the rhizome of *C. longa* and reported the presence of several compounds with biological activity and medicinal properties (21). *C. longa* is a significant constituent of many pharmaceutical, healthcare and cosmetic products due to its immense medicinal potential. However, *C. caseia* remains comparatively less

**Table 1.** Identified phytocompounds in GC-MS analysis of methanolic extract of rhizome of *C. longa.*

| Peak | R. Time | Area% | RI Value | Name |
|---|---|---|---|---|
| 1 | 4.684 | 0.50 | 1002 | Eucalyptol |
| 2 | 5.573 | 0.29 | 1053 | Cyclohexene,1-Methyl-4-(1-Methylethylid) |
| 3 | 9.516 | 0.31 | 1296 | Cyclohexene,4-Ethenyl-4-Methyl-3-(1-Methylethenyl)-1 |
| 4 | 10.195 | 0.09 | 1344 | Cyclohexane,1-Ethenyl-1-Methyl-2,4-Bis |
| 5 | 10.302 | 1.32 | 1351 | Cyclohexane,1-Ethenyl-1-Methyl-2,4-Bis(1-Methylethenyl) |
| 6 | 10.430 | 0.08 | 1360 | (1s,5s)-2-Methyl-5-((R)-6-Methylhept-5-En-2-Yl) Bicyclo (7-epi-Sesquithujene) |
| 7 | 10.490 | 0.05 | 1364 | Bergamotol, Z- Alpha. -Trans |
| 8 | 10.752 | 1.04 | 1383 | Caryophyllene |
| 9 | 10.840 | 1.73 | 1389 | Gamma. - Elemene |
| 10 | 11.090 | 0.19 | 1407 | (E)- Beta. - Famesene |
| 11 | 11.134 | 0.09 | 1410 | (1s,5s)-4-Methylene-1-((R)-6-Methylhept-5-En-2-Yl) Bicyc (Sesquisabinene) |
| 12 | 11.236 | 0.08 | 1418 | 1,4,8-Cycloundecatriene,2,6,6,9-Tetramethy (Alpha-Humulen) |
| 13 | 11.518 | 0.17 | 1439 | 1-(1,5-Dimethyl-4-Hexenyl)-4-Methylbenzen (ALPHA-CURCUMEN) |
| 14 | 11.569 | 0.51 | 1443 | 1,6-Cyclodecadiene,1-Methyl-5-Methylene- (GERMACRENE-D) |
| 15 | 11.684 | 6.82 | 1452 | (1s,5s)-2-Methyl-5-((R)-6-Methylhept-5-En-2 Yl) Bicyclo (7-epi-Sesquithujene) |
| 16 | 11.849 | 0.73 | 1464 | Beta. -Bisabolene |
| 17 | 12.067 | 6.03 | 1481 | Cyclohexene,3-(1,5-Dimethyl-4-Hexenyl)-6-Methylene |
| 18 | 12.197 | 0.13 | 1490 | 5-Isopropyl-2-Methylbicyclo [3.1.0] Hex-3-En (THUJOL) |
| 19 | 12.466 | 0.20 | 1521 | (1s,2r,5r)-2-Methyl-5-((R)-6-Methylhept-5-En-2-Yl) Bicyc |
| 20 | 12.513 | 0.21 | 1516 | 1,6,10-Dodecatrien-3-Ol,3,7,11-Trimethyl-,(E)- (trans-Nerolidol) |
| 21 | 12.571 | 0.82 | 1520 | GermacreneB |
| 22 | 12.694 | 0.09 | 1530 | Tumerone |
| 23 | 12.923 | 0.79 | 1549 | Trans-Sesquisabinene Hydrate |
| 24 | 12.980 | 0.21 | 1554 | 3,7-Cyclodecadien-1-One,3,7-Dimethyl-10-(1-Methylethyli (Germacron) |
| 25 | 13.047 | 7.07 | 1559 | Beta. - Elemenone |
| 26 | 13.203 | 1.37 | 1572 | Zingiberenol |
| 27 | 13.350 | 1.22 | 1584 | Iso Curcumenol |
| 28 | 13.415 | 0.58 | 1589 | Zingiberenol |
| 29 | 13.475 | 0.32 | 1594 | Epi-Alpha-Patschulene |
| 30 | 13.545 | 0.10 | 1559 | 4-(Bicyclo [3.2.0] Hept-3-En-2-Yloxy) Bicyclo |
| 31 | 13.653 | 0.10 | 1609 | Iso Curcumenol |
| 32 | 13.728 | 2.13 | 1651 | Benzene,1-(1,5-Dimethyl-4-Hexenyl)-4-Methy (ALPHA-CURCUMEN) |
| 33 | 13.880 | 32.72 | 1628 | Tumerone |
| 34 | 14.088 | 1.22 | 1646 | (4r,6s)-2-Methyl-6-((S)-4-Methylenecyclohex-2-En-1-Yl) H |
| 35 | 14.246 | 17.71 | 1660 | Curlone |
| 36 | 14.449 | 0.19 | 1677 | Curcuphenol ` |
| 37 | 14.573 | 0.48 | 1688 | Curcumenol |
| 38 | 14.720 | 1.00 | 1701 | (6r,7r)-Bisabolone |
| 39 | 14.854 | 0.12 | 1713 | Tumerone |
| 40 | 14.926 | 0.24 | 1719 | 1,5-Heptadien-4-One,3,3,6-Trimethyl- |
| 41 | 15.049 | 0.27 | 1730 | (E)-Atlantone |
| 42 | 15.169 | 2.44 | 1742 | Cyclohexane,3,4-Bis(1-Methylethenyl)-1,1-D |
| 43 | 15.274 | 0.98 | 1751 | (Z)-. Gamma. -Atlantone |
| 44 | 15.427 | 0.06 | 1765 | Picrotoxinin |

| | | | | |
|---|---|---|---|---|
| 45 | 15.482 | 0.22 | 1770 | PeritylTiglate,Trans- |
| 46 | 15.742 | 0.21 | 1794 | 4,8a-Dimethyl-6-(2-Methyl-2-Oxiranyl)-4a,5,6 |
| 47 | 15.822 | 3.30 | 1801 | FragranylAcetate |
| 48 | 16.492 | 0.26 | 1865 | HexadecanoicAcid,MethylEster |
| 49 | 17.347 | 0.07 | 1949 | 1-Naphthalene carboxylic Acid, Decahydro |
| 50 | 18.128 | 0.29 | 2029 | 9,12-Octadecadienoic Acid(Z, Z)-,MethylEster |
| 51 | 18.187 | 0.29 | 2035 | 9-Octadecenoic Acid, MethylEster,(E)- |
| 52 | 18.423 | 0.08 | 2059 | MethylStearate |
| 53 | 19.955 | 0.22 | 2226 | GlycidylPalmitate |
| 54 | 21.656 | 0.38 | 2397 | 1,8,11-Heptadecatriene,(Z, Z)- |
| 55 | 21.710 | 0.51 | 2401 | GlycidylOleate |
| 56 | 22.005 | 0.14 | 2424 | GlycidylPalmitate |
| 57 | 22.400 | 0.11 | 2455 | 1,2-BenzenedicarboxylicAcid |
| 58 | 23.496 | 0.12 | 2532 | Propane,1,2-Dimethoxy-3-[(2-Methoxyhexadecyl) Oxy] |
| 59 | 24.112 | 0.20 | 2571 | Cyclopropane,1,1-Dichloro-2,2,3,3-Tetramethyl- |
| 60 | 25.084 | 0.67 | 2631 | 9,12-Octadecadienoic Acid (Z, Z)-,2-Hydroxy-1- (Hydroxym) (.beta.-Monolinolein) |
| 61 | 27.335 | 0.17 | 2795 | 1,3-Dioxolane,4-[(2-Methoxyhexadecyl) Oxy] |
| | | 100.00 | | |

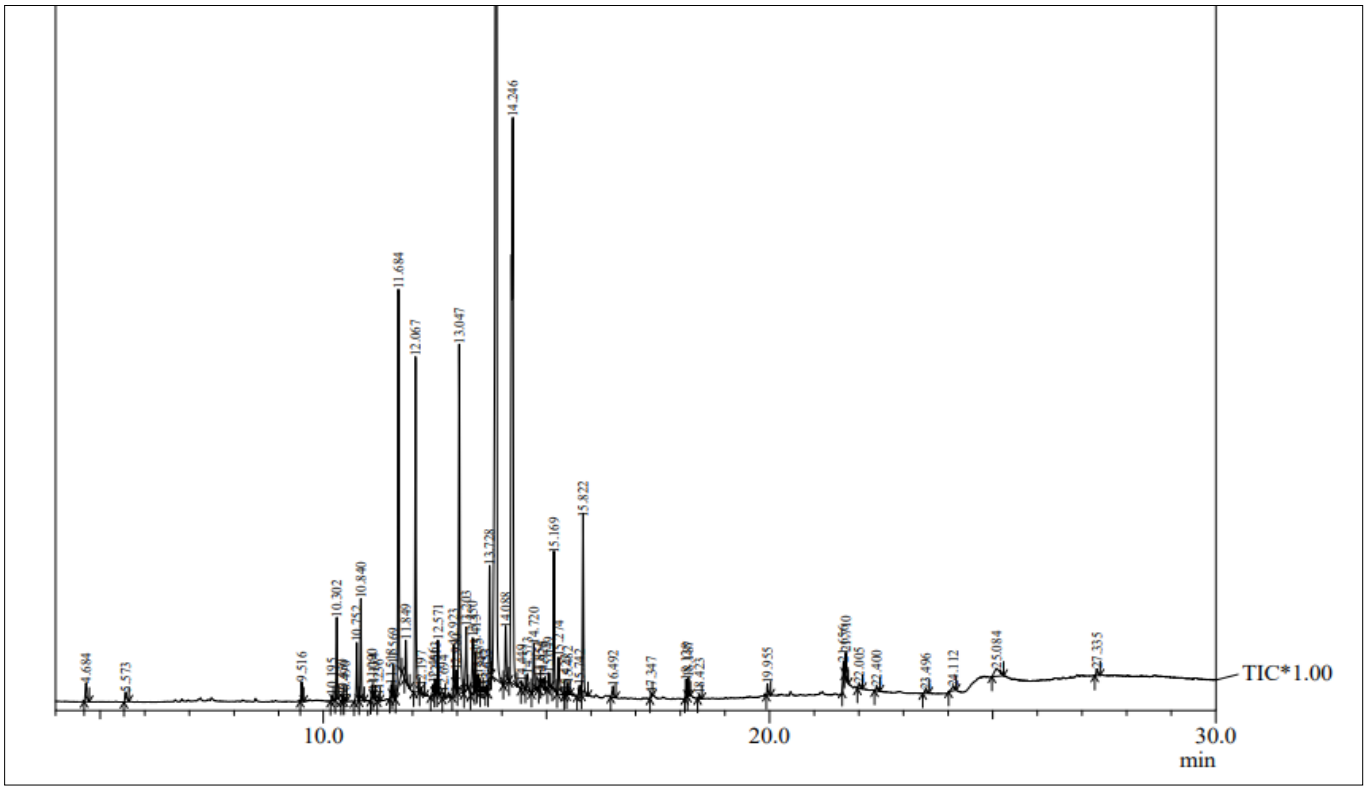


**Fig. 2.** GC-MS analysis of methanolic extract of rhizome of *C. longa.*

explored on a commercial or industrial scale. As evidenced by the FTIR and GC-MS results of the present study, *C. caseia* synthesises a wide range of phytoconstituents with various biological activities. This underscores the need for further research, including clinical trials, to validate the utilization of *C. caesia* in the food, cosmetic and healthcare industries on a commercial scale.

Most of the reported literature focuses on the medicinal potential of turmeric rhizomes. However, in the present study, the GC-MS analysis of the leaves of *C. longa* and *C. caesia* was also conducted to identify the phytocompounds present in the leaves of both species (Table 3, Fig. 4). The major compounds found in the extract of *C. caesia* leaves include L-α-terpineol, curcumenone, 2-myristynoyl pantetheine, oleic acid, cis-vaccenic acid, 6-octadecanoic acid, 1-heptatriacotanol and tridecanediol. Similarly, the major phytocompounds found in the leaf extract of *C. longa* were n-hexadecanoic acid, cis-vaccenic acid, 2-myristynoyl pantetheine, 6-octadecanoic acid, cis-11-eicosenoic acid, cis-vaccenic acid, palmitoyl chloride, diosgenin and tridecanediol.

In an earlier study conducted (22), 11 phytocompounds were identified in the leaf extract of *C. longa*, including hexadecanoic acid and cis-vaccenic acid, which were also found in the leaf extract in the present study. Many of the compounds identified in the leaves of the 2 turmeric species are well known for their biological activities. Terpineol possesses antioxidant and anticancer properties, diosgenin is a natural antioxidant known for its neuropro-

**Table 2.** Identified phytocompounds in GC-MS analysis of methanolic extract of rhizome of *C. caseia.*

| Peak | R. Time | Area% | RI Value | Name |
|---|---|---|---|---|
| 1 | 4.685 | 3.17 | 1002 | Eucalyptol |
| 2 | 6.679 | 2.92 | 1119 | (+)-2-Bornanone (Camphor) |
| 3 | 6.968 | 0.95 | 1136 | Bicyclo [2.2.1] Heptan-2-Ol,1,7,7-Trimethyl- (exo-2-Camphanol) |
| 4 | 7.118 | 0.30 | 1145 | endo-Borneol (Baros camphor) |
| 5 | 7.253 | 0.21 | 1153 | Phosphonic Acid, Dioctadecyl Ester |
| 6 | 7.387 | 0.15 | 1161 | Decane,6-ethyl-2-methyl- |
| 7 | 7.501 | 0.22 | 1168 | 3-Cyclohexene-1-Methanol, Alpha., Alpha., |
| 8 | 8.068 | 0.22 | 1202 | Cyclohexane, Hexyl |
| 9 | 8.829 | 1.98 | 1251 | Isobornylacetate |
| 10 | 9.514 | 0.62 | 1296 | Cyclohexene,4-Ethenyl-4-Methyl-3-(1-Methylethenyl) |
| 11 | 9.896 | 0.16 | 1323 | Dodecane,6-cyclohexyl- |
| 12 | 10.193 | 0.21 | 1343 | 2,4-Diisopropenyl-1-Methyl-1-Vinylcyclohe |
| 13 | 10.255 | 0.49 | 1348 | 1-Tetradecene |
| 14 | 10.300 | 2.98 | 1351 | Cyclohexane,1-Ethenyl-1-Methyl-2,4-Bis |
| 15 | 10.355 | 0.30 | 1355 | Dodecane |
| 16 | 10.749 | 0.92 | 1383 | Caryophyllene |
| 17 | 10.839 | 0.89 | 1389 | gamma. -Elemene |
| 18 | 11.442 | 0.27 | 1433 | 4a,8-Dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahyd |
| 19 | 11.568 | 1.40 | 1443 | 1,6-Cyclodecadiene,1-Methyl-5-Methylene (gamma.-Amorphene) |
| 20 | 11.680 | 0.17 | 1452 | 2-Bromododecane |
| 21 | 11.717 | 0.53 | 1454 | Benzofuran,6-Ethenyl-4,5,6,7-Tetrahydro-3,6-Dimethyl-5-Is (Curzerene) |
| 22 | 11.770 | 0.22 | 1458 | Neoalloocimene |
| 23 | 12.006 | 0.25 | 1476 | Naphthalene,1,2,3,5,6,8a-Hexahydro-4,7-Dimethyl-1 (.delta.-Cadinene) |
| 24 | 12.570 | 0.49 | 1520 | GermacreneB |
| 25 | 12.823 | 1.46 | 1541 | 1-Heptadecene |
| 26 | 12.958 | 1.43 | 1552 | 1H-Cycloprop[E]Azulen-4-Ol, Decahydro-1,1,4,7-Tetramethy ((+)-Viridiflorol) |
| 27 | 13.071 | 14.86 | 1561 | Epicurzerenone (5-epi-Curzerenone) |
| 28 | 13.347 | 2.58 | 1583 | 5.Beta. -Guaia-7(11),10(14)-Dien-8. Alpha. -Ol,5, |
| 29 | 13.444 | 0.24 | 1591 | Benzene,(1-propylheptadecyl)- |
| 30 | 13.644 | 0.40 | 1608 | 1-Heptatriacotanol |
| 31 | 13.791 | 1.65 | 1621 | Bicyclo [6.3.0] Undec-1(8)-En-3-Ol,2,2,5,5-Tetramethyl |
| 32 | 14.198 | 0.78 | 1656 | 3,7-Cyclodecadien-1-One,3,7-Dimethyl-10-(1-Methylethyli (Germacron) |
| 33 | 14.279 | 0.37 | 1663 | 1h-Benzocyclohepten-7-Ol,2,3,4,4a,5,6,7,8-Oct |
| 34 | 14.456 | 0.24 | 1678 | 2-Isopropylidene-3-methylhexa-3,5-dienal |
| 35 | 14.570 | 2.32 | 1688 | Curcumenol |
| 36 | 14.765 | 2.19 | 1705 | 12.Alpha. - D-5. Alpha. -Androstan-11-One |
| 37 | 14.911 | 0.42 | 1718 | 9-Heptadecanone |
| 38 | 15.098 | 1.26 | 1735 | 1-Octadecene |
| 39 | 15.162 | 6.62 | 1741 | Z, Z-3,15-Octadecadien-1-OlAcetate |
| 40 | 15.280 | 0.83 | 1752 | beta. -Cyclocostunolide |
| 41 | 15.381 | 0.30 | 1761 | 2-HeptenedioicAcid,4-Cyclopropyl-,DimethylEster,(E) |
| 42 | 15.431 | 0.30 | 1765 | Ambrial |
| 43 | 15.741 | 0.96 | 1793 | Curcumenone |
| 44 | 15.820 | 0.70 | 1801 | Fragranyl Isobutyrate |

| | | | | |
|---|---|---|---|---|
| 45 | 15.915 | 1.72 | 1810 | Methyl2-(1,1-Dimethyl-5-Oxohexyl)-2-Cyclo |
| 46 | 16.336 | 1.14 | 1850 | 2,3,3-Trimethyl-2-(3-Methylbuta-1,3-Dienyl)-6-Methylenec |
| 47 | 16.491 | 0.37 | 1865 | Hexadecanoic acid, methylester |
| 48 | 17.145 | 0.41 | 1929 | 1-Nonadecene |
| 49 | 17.447 | 1.04 | 1959 | Zederone |
| 50 | 17.522 | 0.48 | 1967 | 4-(5,5-Dimethylspiro [2.5] Oct-4-Yl)-3-Buten-2-O |
| 51 | 17.611 | 0.34 | 1975 | 1h-Cycloprop[E]Azulen-4-Ol,Decahydro-1,1, |
| 52 | 17.837 | 0.21 | 1998 | Cyclodecanone |
| 53 | 18.127 | 1.07 | 2028 | 9,12-Octadecadienoic Acid(Z, Z)-,Methyl Ester |
| 54 | 18.184 | 2.03 | 2034 | 9-OctadecenoicAcid, Methyl Ester,(E) |
| 55 | 18.418 | 0.23 | 2059 | MethylStearate |
| 56 | 18.905 | 0.19 | 2111 | Cyclonona Siloxane, Octadeca Methyl |
| 57 | 19.014 | 0.24 | 2122 | Penta Fluoro Propionic Acid, TetradecylEster |
| 58 | 19.952 | 1.35 | 2225 | Glycidyl Palmitate |
| 59 | 20.466 | 0.65 | 2281 | 2,5-Di (Trifluoromethyl)BenzoicAcid,3-HexadecylEster |
| 60 | 20.724 | 1.91 | 2308 | (E)-Labda-8(17),12-Diene-15,16-Dial |
| 61 | 21.183 | 0.24 | 2352 | OleoylChloride |
| 62 | 21.654 | 2.27 | 2396 | 1,8,11-Heptadecatriene,(Z, Z) |
| 63 | 21.705 | 3.19 | 2401 | GlycidylOleate |
| 64 | 21.867 | 5.47 | 2414 | 19,19-Dimethoxy-3-Oxoandrost-1-En-17-YlAc |
| 65 | 21.943 | 0.54 | 2419 | 1H-Dibenzo, Fluorene,13-(Decahydro-1-Naphthalenyl) |
| 66 | 22.001 | 0.37 | 2424 | GlycidylPalmitate |
| 67 | 22.110 | 0.50 | 2432 | Dihydro-Isosteviol MethylEster |
| 68 | 23.489 | 0.77 | 2532 | 1,3-Dioxolane,4-[(2-Methoxyhexadecyl) Oxy] |
| 69 | 24.093 | 3.77 | 2570 | Linolool Oxide, TMS Derivative |
| 70 | 25.102 | 7.05 | 2632 | 9,12-OctadecadienoicAcid(Z, Z)-,2-Hydroxy-1- (Hydroxym) |
| 71 | 27.332 | 1.28 | 2795 | MalonicAcid,2,4-Dimethylpent-3-YlUndecylEster |
| 72 | 30.959 | 1.23 | 3052 | Propane,1,2-Dimethoxy-3-[(2-Methoxyhexadecyl) Oxy] |
| | | 100.00 | | |

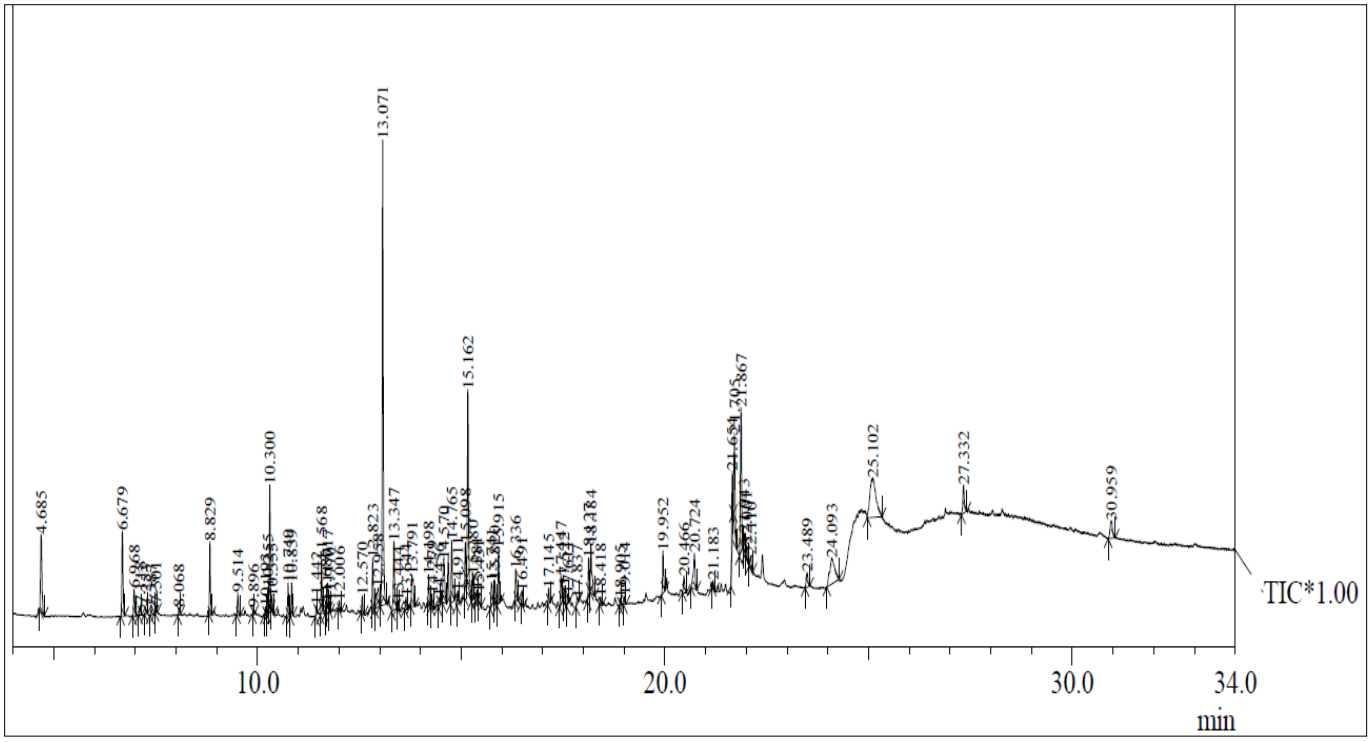


**Fig. 3.** GC-MS analysis of methanolic extract of rhizome of *C. caseia.*

tective

**Table 3.** Major phytocompounds identified to be present in leaves of *C. caseia* and *C. longa*.

| R.T | Area % | RI | Name of compound |
|---|---|---|---|
| ***C. caseia*** | | | |
| 4.27 | 10.06 | 1208 | Ketone, 2,2-dimethyl cyclohexyl methyl |
| 8.22 | 9.11 | 1130 | L-à-Terpineol |
| 22.23 | 2.57 | 1806 | 2(3H)-Benzofuranone, 6-ethenylhexahydro-3,6-dimethyl-7-(1-methylethenyl)-, [3S-(3à,3aà,6à,7á,7aá)]- |
| 23.47 | 1.82 | 1844 | Curcumenone |
| 29.5 | 29.79 | 2141 | Oleic Acid |
| 34.74 | 1.28 | 2066 | Methyl 8,9-octadecadienoate |
| 34.85 | 3.84 | 2116 | cis-Vaccenic acid |
| 34.97 | 0.92 | 1506 | Tridecanedial |
| 35.48 | 1.67 | 2199 | 17-Octadecynoic acid |
| 36.21 | 0.86 | 1529 | 1,13-Tetradecadien-3-one |
| 37.56 | 1.03 | 1506 | Tridecanedial |
| 38.29 | 5.95 | 2073 | 6-Octadecenoic acid |
| 38.51 | 1.35 | 2103 | 1,2-15,16-Diepoxyhexadecane |
| 40.83 | 4.14 | 1698 | Pumilitoxin 251d |
| 41.04 | 7.50 | 3942 | 1-Heptatriacotanol |
| 41.64 | 3.33 | 2076 | 12-Methyl-E,E-2,13 - octadecadien-1-ol |
| 45.83 | 3.11 | 3057 | 24-Noroleana-4(23),12-diene, 3-methyl-, (3à)- |
| ***C. longa*** | | | |
| 26.17 | 7.36 | 1961 | n-Hexadecanoic acid |
| 29.49 | 23.70 | 2116 | cis vaccenic acid |
| 34.83 | 2.42 | 2163 | trans-13-Octadecenoic acid |
| 35.84 | 1.54 | 1506 | Tridecanedial |
| 37.18 | 1.62 | 2356 | cis-11-Eicosenoic acid |
| 37.25 | 1.15 | 1936 | erythro-(cis)(1,4), (cis)(1',4')-4,4'-Dihydroxybicyclooctyl |
| 37.65 | 2.24 | 2356 | cis-11-Eicosenoic acid |
| 38.29 | 7.45 | 2073 | 6-Octadecenoic acid |
| 38.64 | 1.24 | 2116 | cis-Vaccenic acid |
| 38.68 | 1.67 | 1102 | Cyclooctane carboxaldehyde |
| 39.56 | 1.24 | 1731 | Cyclohexadecanone |
| 41.69 | 4.14 | 2600 | Eicosanenitrile |
| 41.93 | 8.28 | 3220 | Diosgenin |
| 43.04 | 2.93 | 1632 | Z-12-Tetradecenal |
| 44.37 | 3.24 | 975 | 6-Butyl-2-methyl-1,3,6,2-dioxaza phosphocane-2-oxide |

effect and cis-vaccenic acid has antibacterial and hypolipidemic effects. This diverse phytochemical richness of turmeric leaves indicates their potential for medicinal applications similar to turmeric rhizomes. Further exploration of turmeric leaves for their medicinal applications is warranted.

## *Analysis of biological activities*

### Antioxidant activity

The antioxidant activity of methanolic extracts from the rhizome of *C. longa* and *C. caseia*, along with BHT (Butylated Hydroxytoluene), ascorbic acid and rutin, was determined in terms of DPPH scavenging activity. The results indicated significant DPPH scavenging activity for both test samples and standards. The rhizome and leaf extracts of *C. caesia* and *C. longa* exhibited notable antioxidant potential, comparable to the standards rutin, BHT and ascorbic acid. Both plant samples and standards showed an increase in antioxidant potential as the concentration increased from 25 µg/mL to 50 µg/mL and 75 µg/mL (Fig. 5), with the maximum activity observed at 75 µg/mL. At this concentration, the antioxidant activity of the rhizomes of *C. caesia* and *C. longa* was found to be 85.35 % and 86.3 % respectively. Similarly, the leaf extracts of both species exhibited comparable antioxidant activity at 75 µg/mL, with *C. longa* at 84.09 % and *C. caesia* at 83.45 %. The results indicate that the antioxidant activity of the rhizomes and leaves of *C. caesia* and *C. longa* is comparable to that of the standard compounds at a

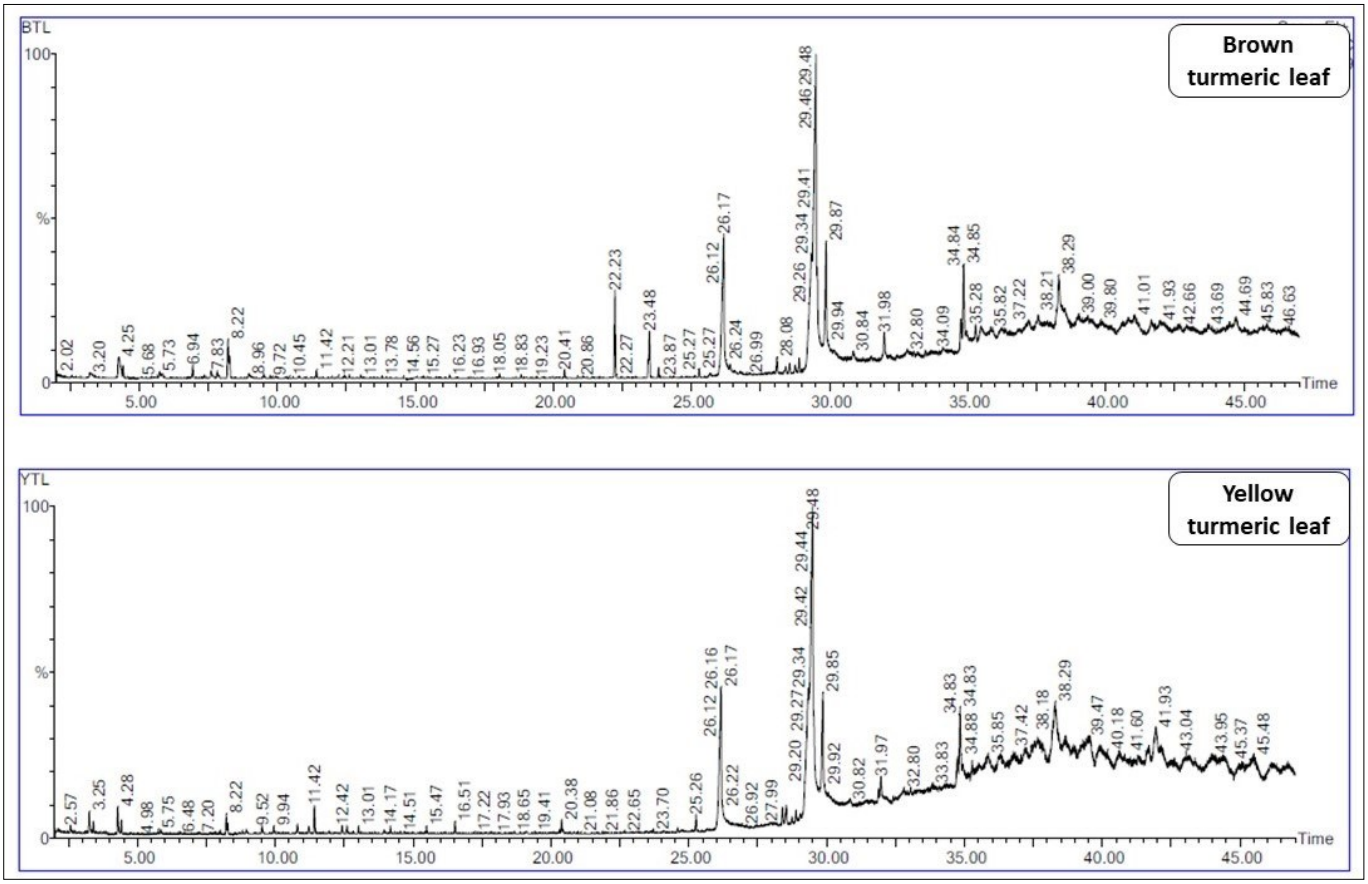


**Fig. 4.** GC-MS analysis of methanolic extract of leaf of *C. caseia* (brown turmeric) and *C. longa* (yellow turmeric).

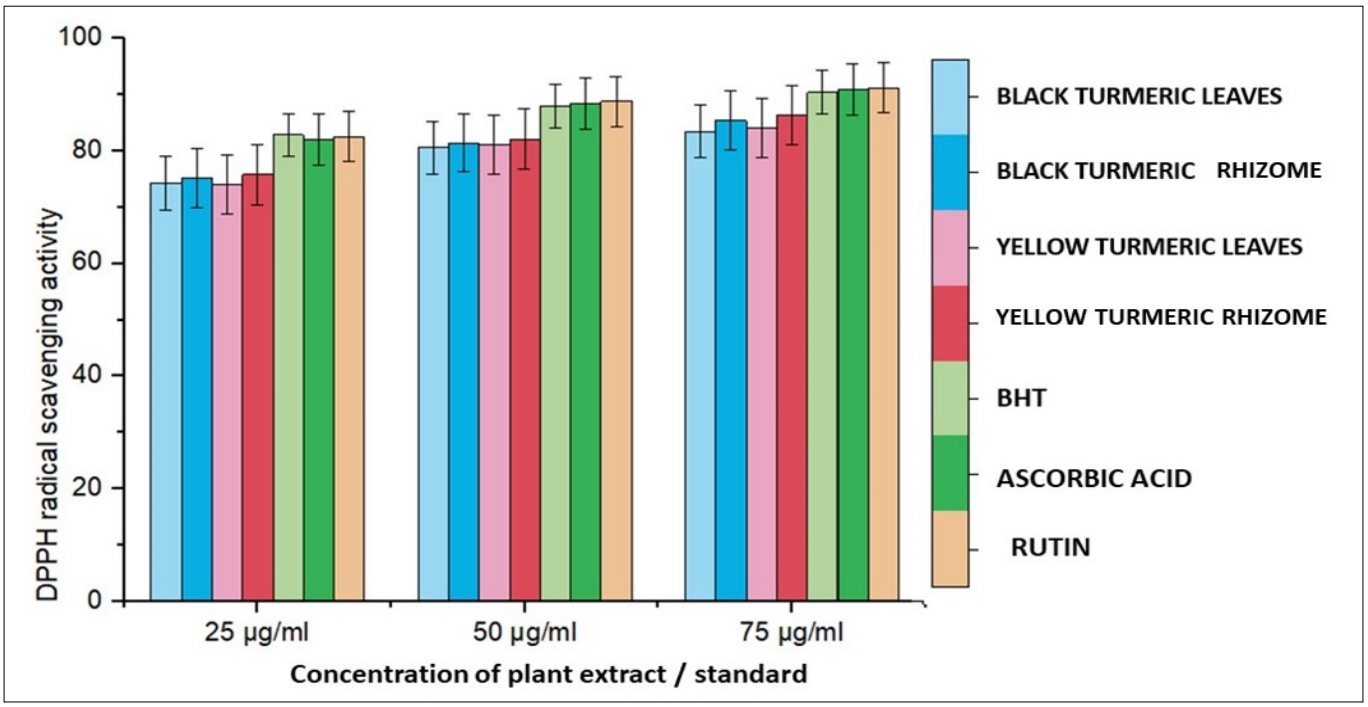


**Fig. 5.** Anti-oxidant activity of methanolic extract of rhizome and leaves of *C. caseia* and *C. longa*.

concentration of 75 µg/mL, with acetylsalicylic acid, ascorbic acid and rutin exhibiting antioxidant activity of 90.29 %, 90.81 %, and 91.07 % respectively. This supports the potential of not only the rhizomes but also the leaves of both turmeric species as a source of natural antioxidants. When comparing the antioxidant activity of the 2 turmeric species, the leaves and rhizomes of *C. longa* exhibited higher antioxidant potential compared to those of *C. caesia*. The antioxidant potential of the leaves and rhizomes of *C. caesia* and *C. longa* is attributed to the presence of phenolic and other antioxidant compounds, as revealed by GC -MS analysis. Other studies have also supported the utilization of turmeric species for the development of antioxidant drugs (2).

Researchers conducted a comparative study to evaluate anti-oxidant potential of *C. longa, C. caesia* and *C. aromatic* (23). They reported that all 3 species exhibited potent anti-oxidant activity, with the maximum activity

observed in *C. aromatica.* Most applications of turmeric in industries, medicines and healthcare are limited to rhizomes; however, the findings of the present study indicate the inherent potential of turmeric leaves for medicinal applications. Earlier studies also support the utilization of *C. longa* rhizomes and leaves as a source of natural antioxidants and their potential use in the food industries (24, 25).

### Anti-inflammatory activity

The anti-inflammatory activity of *C. longa* and *C. caesia* rhizome extracts, as well as aspirin, acetylsalicylic acid and diclofenac potassium, was measured in terms of the percentage inhibition of albumin denaturation. Similar to the antioxidant activity, the anti-inflammatory activity increased with the concentration of the plant extract and standard, ranging from 25 µg/mL to 50 µg/mL and 75 µg/mL. The maximum anti-inflammatory activity at 75 µg/mL was exhibited by *C. caesia* rhizome (90.12 %), followed by *C. longa* rhizome (89.77 %), *C. caesia* leaves (87.12 %) and *C. longa* leaves (86.77 %). The anti-inflammatory activity of the leaves and rhizomes of *C. caesia* and *C. longa* was found to be comparable to that of the standard compounds. At a concentration of 75 µg/mL, the anti-inflammatory activity of aspirin, acetylsalicylic acid and diclofenac was found to be 93.2 %, 91.71 % and 94.14 % respectively (Fig. 6). The anti-inflammatory potential of the leaves of *C. longa* and *C. caesia* has been less explored. The results of the present study suggest that both the rhizomes and leaves of these turmeric species can be further investigated as natural sources of anti-inflammatory compounds. In a prominent earlier study conducted (26), the anti-inflammatory activity of *C. longa* rhizome and diclofenac was reported, with a reduction in the paw size of rats under the influence of the rhizome and diclofenac. Further studies can be conducted to validate the therapeutic potential of the leaves of *C. longa* and *C. caesia*.

## Conclusion

*C. longa* has been cultivated for centuries due to its significant medicinal potential and has been a crucial ingredient in traditional as well as industrial products. However, its related species, *C. caesia*, remains comparatively underutilized for medicinal (both traditional and modern) and industrial purposes. Furthermore, the lack of large-scale commercial cultivation of *C. caesia* further hinders its utilization. There is a pressing need to raise awareness among local stakeholders to foster integrated efforts between cultivators and industries (including herbal products, food, cosmetics, and pharmaceuticals) to recognize *C. caesia* as a valuable medicinal plant. The present study highlights the medicinal potential of *C. caesia*, which is comparable to *C. longa* in terms of the presence of bioactive metabolites and biological activities. The antioxidant and anti-inflammatory activities observed in both species suggest their potential use as natural sources of antioxidant compounds. These compounds could potentially replace synthetic antioxidants in cosmetic, food and pharmaceutical industries. Similarly, the active compounds of turmeric species can be explored as sources of potent anti-inflammatory agents of natural origin, potentially replacing synthetic anti-inflammatories for the treatment of various inflammatory diseases. Such studies will also support the commercial cultivation of turmeric species on a larger scale, thereby providing economic benefits to local stakeholders and cultivators.

## Acknowledgements

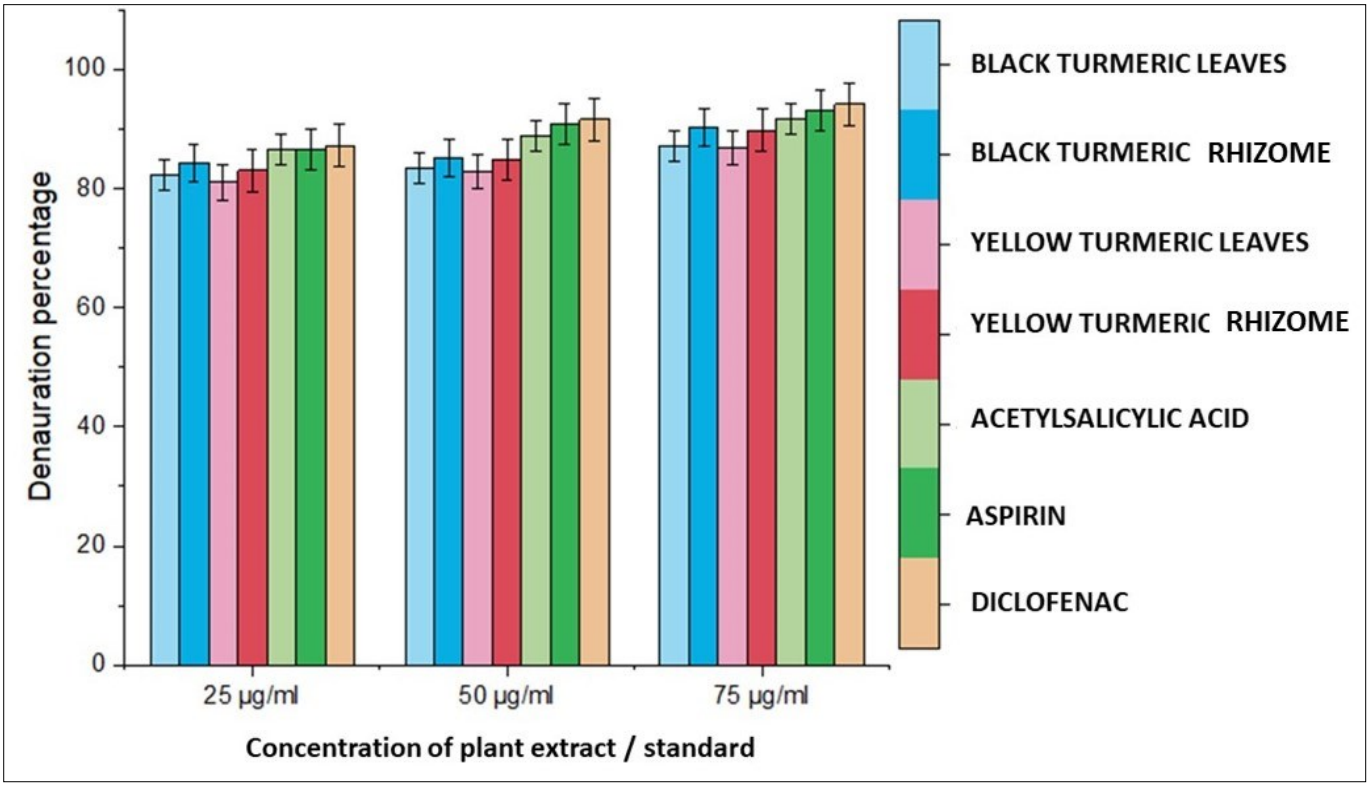


**Fig. 6.** Anti-inflammatory activity of methanolic extract of rhizome and leaves of *C. caseia* and *C. longa.*

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## Authors' contributions

NS and NC identified the research problem, conducted literature survey and designed the experimental study. PC, GS and RS conducted the experimental study. BP and B have contributed for analytical analysis and data interpretation. NK and NC prepared initial draft of manuscript. NS, BP and B have revised the manuscript. All authors approved the final draft of manuscript.

## Compliance with ethical standards

**Conflict of interest**: Authors do not have any conflict of interests to declare.

**Ethical issues**: None.

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