



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 phytochemicals with known medicinal properties in both yellow and black turmeric rhizome. The presence of biologically significant phytochemicals such as methyl stearate, glycidyl oleate, curcumenol and eucalyptol in higher proportion in the rhizome of *C. caesia* compared to yellow turmeric supports further exploration of the commercial and medicinal potential of *C. caesia*. Antioxidant and anti-inflammatory analyses indicated that the rhizome of *C. caesia* 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 phytochemicals 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 °C 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.

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

.....(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:

$$\text{Percentage inhibition (\%)} = \left\{ \frac{(Ab\ c_{nm} - Ab\ s_{nm})}{Ab\ c_{nm}} \times 100 \right\} \dots\dots\dots(\text{Eqn. 2})$$

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 phytochemicals 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).

GC-MS analysis

The GC-MS technique is recognised as an effective approach for identifying various phytochemicals present in plant extracts and other plant products. Once the phytochemicals 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 phytochemicals identified in *C. longa* include picrotoxinin, glycidyl derivatives, (E)-Atlantone, curcuphenol, germacrene B and eucalyptol. Similarly, major phytochemicals found in the rhizome of *C. caesia* include eucalyptol, camphor, isobornyl acetate, viridiflorol, epicurzer-enone, 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 phytochemicals 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 anti-tumour 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 phytochemical in turmeric, exhibiting antibacterial, antifungal and cytotoxic activities (19). Germacrene B has been

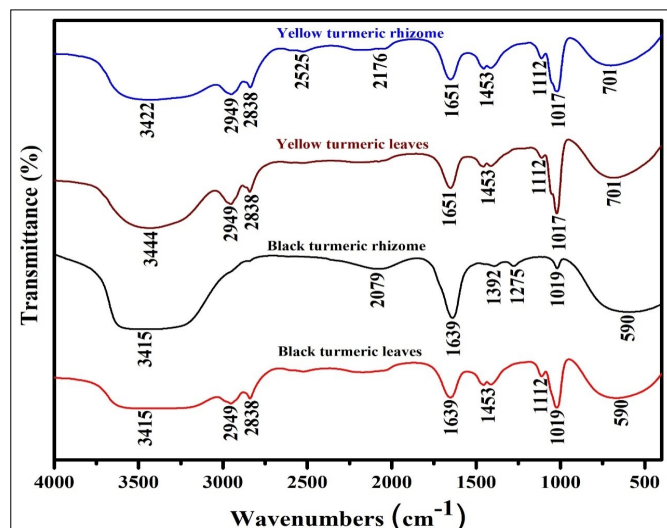


Fig. 1. FTIR analysis of methanolic extract of rhizome and leaves of *C. caseia* and *C. longa*.

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 ex-

Table 1. Identified phytochemicals 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-Humulene)
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-Methylethyl) (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	Curhone
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	PiperitylTiglate,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			

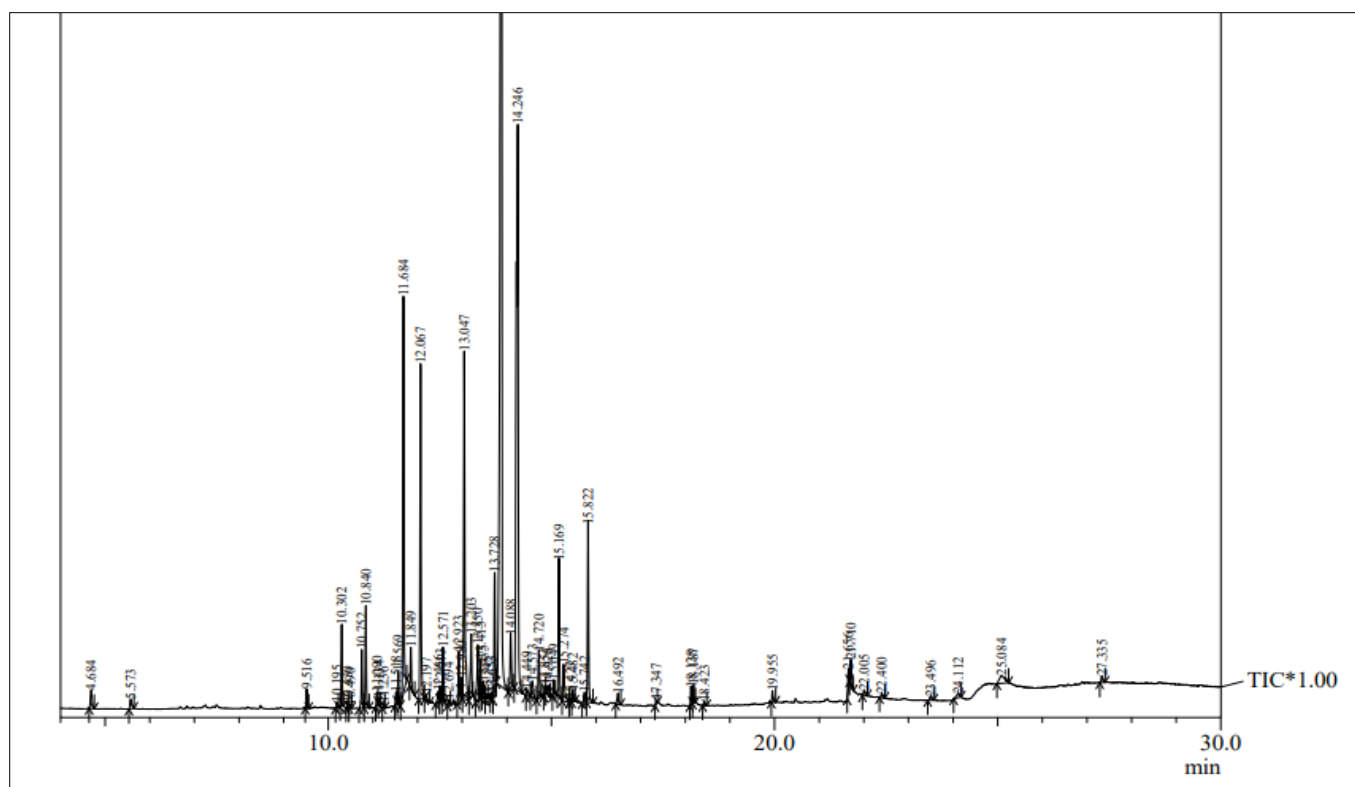


Fig. 2. GC-MS analysis of methanolic extract of rhizome of *C. longa*.

explored on a commercial or industrial scale. As evidenced by the FTIR and GC-MS results of the present study, *C. caesia* 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 phyto-compounds 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 phyto-compounds 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 phytochemicals 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. Terpinol possesses antioxidant and anticancer properties, diosgenin is a natural antioxidant known for its neuroprotective

Table 2. Identified phytochemicals 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	Neoolocimene
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-Tetramethyl ((+)-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-Methylethyl) (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-,Dimethyl(Ester),(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

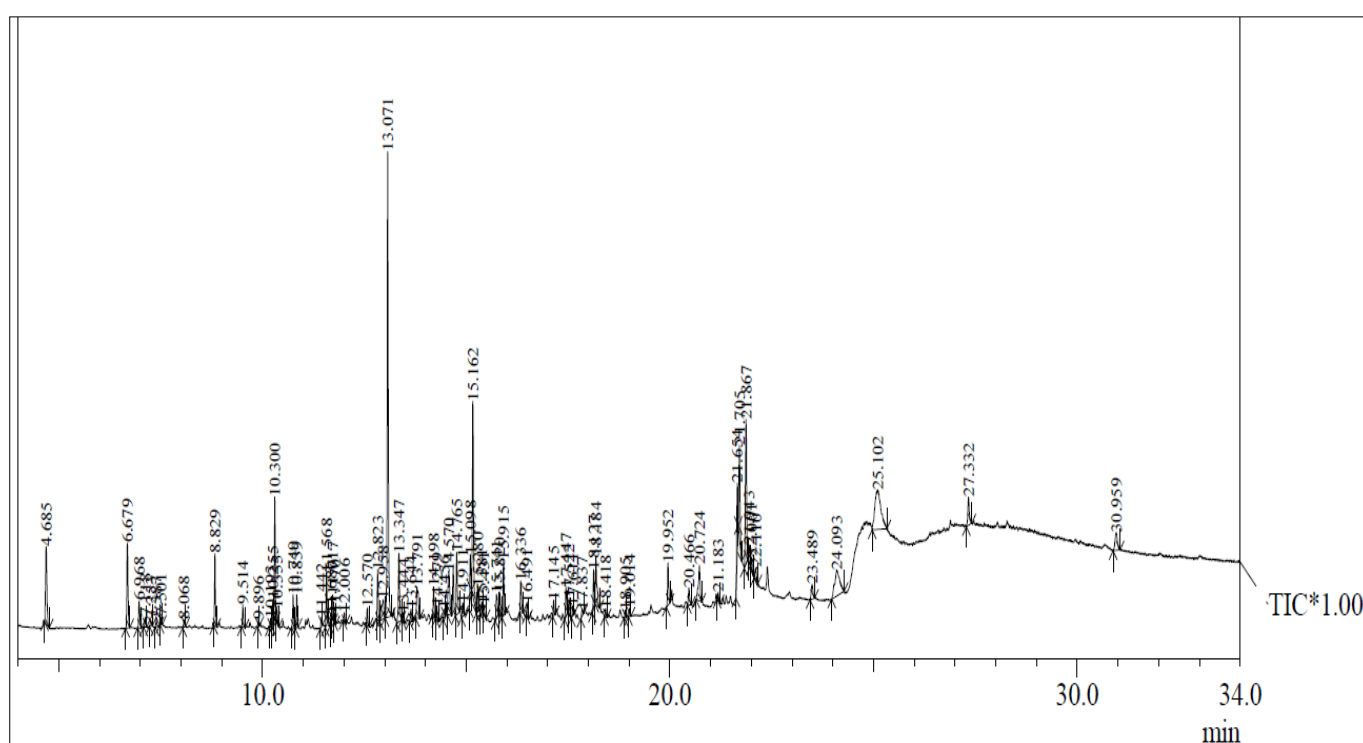


Fig. 3. GC-MS analysis of methanolic extract of rhizome of *C. casea*.

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

R.T	Area %	RI	Name of compound
<i>C. caseia</i>			
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	Pumilotoxin 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)-
<i>C. longa</i>			
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 antioxi-

dant 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 concentration of 75 µg/mL, with acetylsalicylic acid, ascorbic acid and rutin exhibiting antioxidant activity of 90.29 %, 90.81

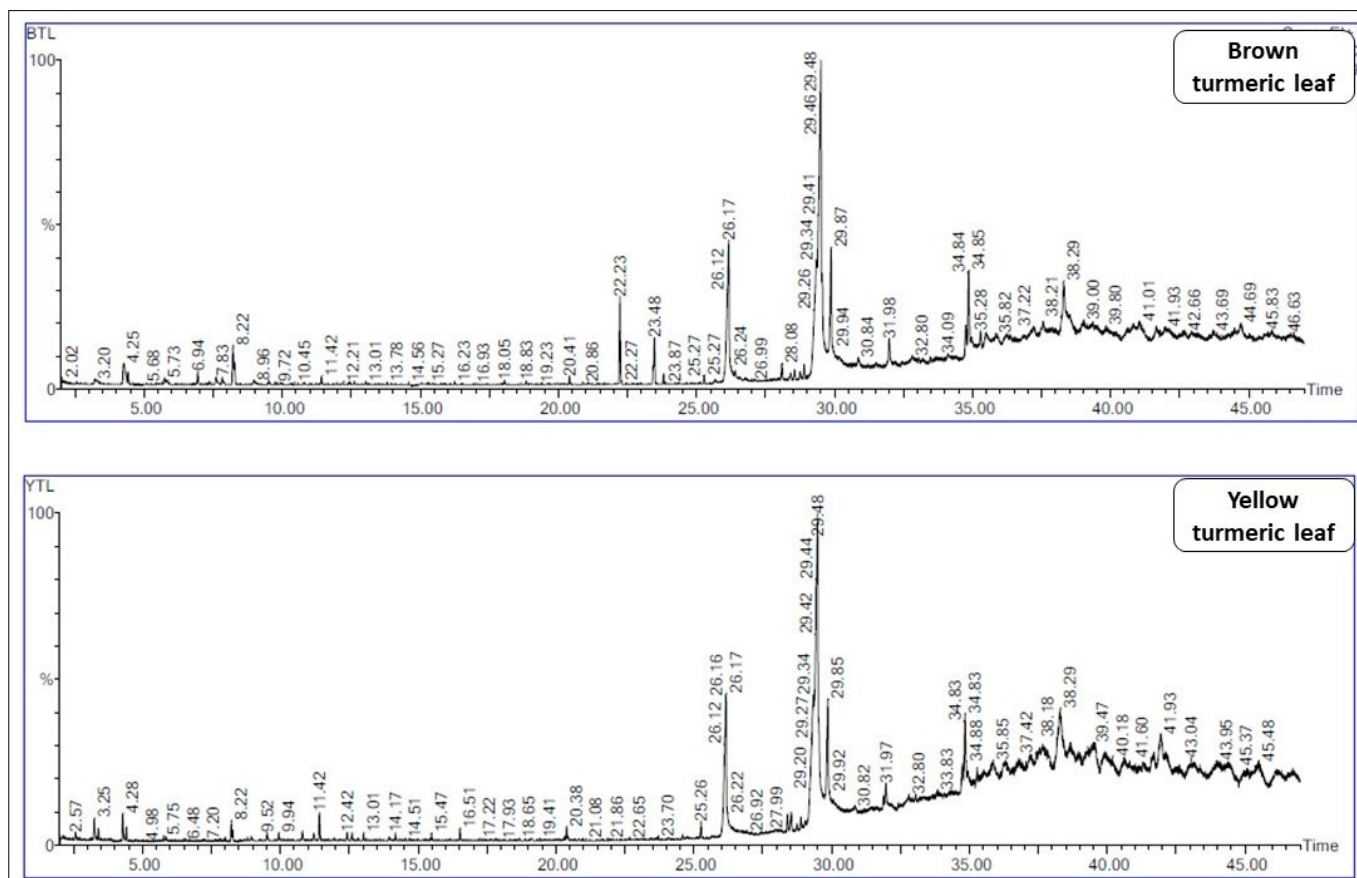


Fig. 4. GC-MS analysis of methanolic extract of leaf of *C. caseia* (brown turmeric) and *C. longa* (yellow turmeric).

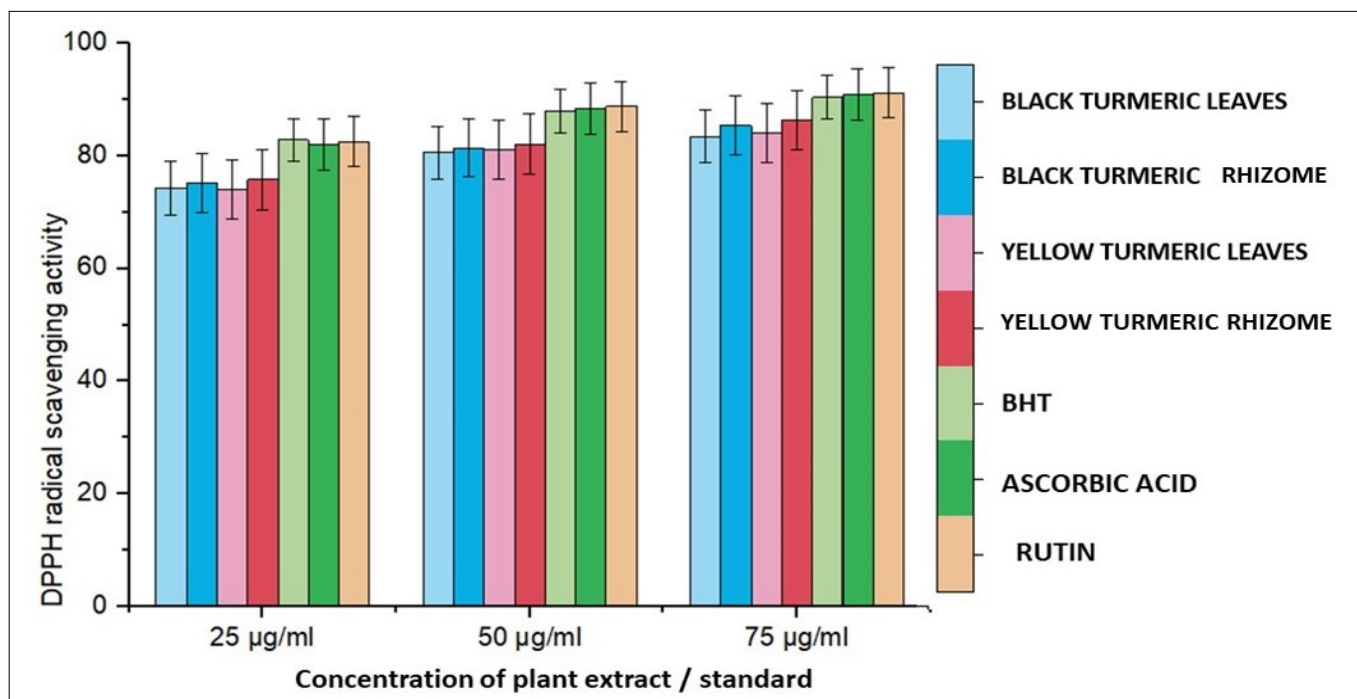


Fig. 5. Anti-oxidant activity of methanolic extract of rhizome and leaves of *C. caseia* and *C. longa*.

%, 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. aromatica* (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 indi-

cate 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 $\mu\text{g}/\text{mL}$ to 50 $\mu\text{g}/\text{mL}$ and 75 $\mu\text{g}/\text{mL}$. The maximum anti-inflammatory activity at 75 $\mu\text{g}/\text{mL}$ 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 $\mu\text{g}/\text{mL}$, 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.

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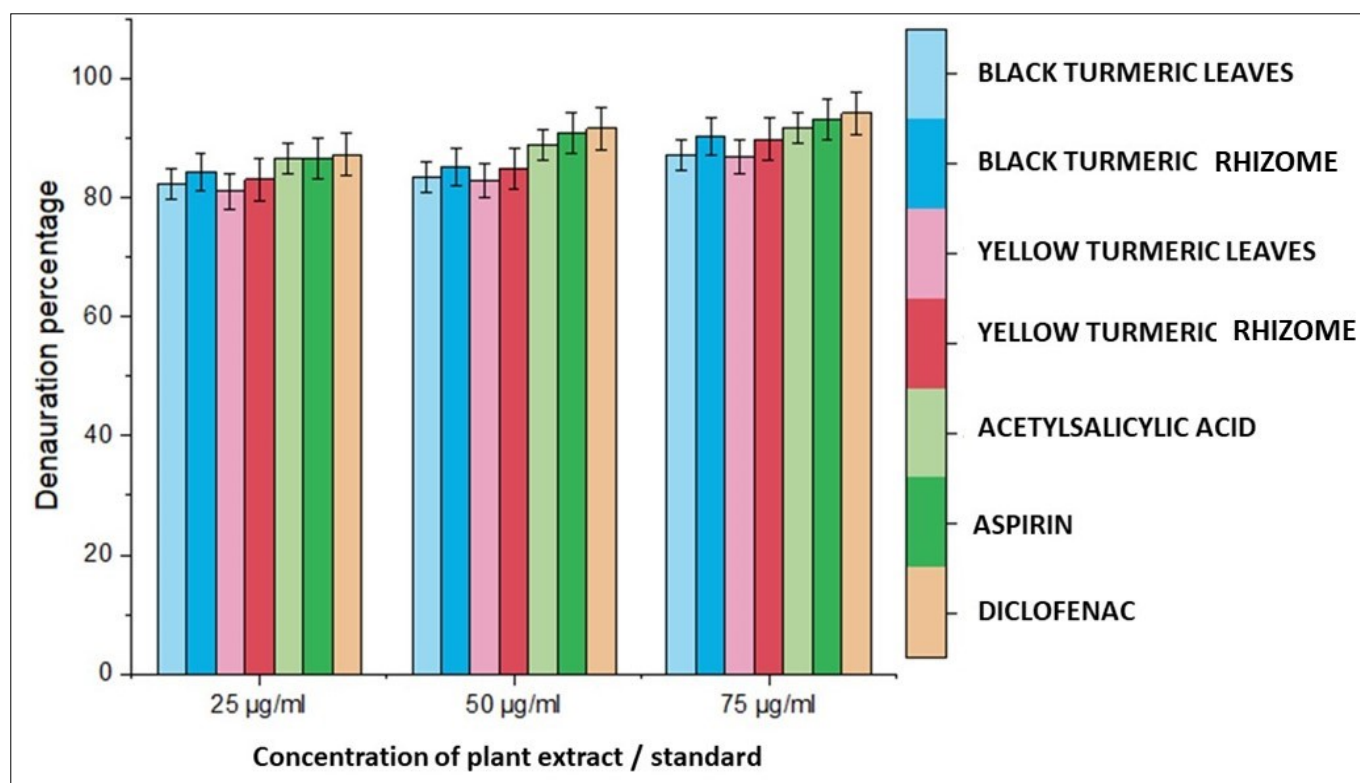


Fig. 6. Anti-inflammatory activity of methanolic extract of rhizome and leaves of *C. caesia* and *C. longa*.

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.

References

- Rohman A, Devi S, Ramadhani D, Nugroho A. Analysis of curcumin in *Curcuma longa* and *Curcuma xanthorrhiza* using FTIR spectroscopy and chemometrics. *Research Journal of Medicinal Plant*. 2015;9(4):179-86. <https://doi.org/10.3923/rjmp.2015.179.186>
- Ahmad RS, Hussain MB, Sultan MT, Arshad MS, Waheed M, Shariati MA, et al. Biochemistry, safety, pharmacological activities and clinical applications of turmeric: A mechanistic review. *Evidence-Based Complementary and Alternative Medicine*. 2020. <https://doi.org/10.1155/2020/7656919>
- Venugopal A, Rinu KA, Dhanish J. Medicinal properties of black turmeric: A review. *Innoriginal: International Journal of Sciences*. 2017;4(3).
- Sahu R, Saxena J. A brief review on medicinal value of *Curcuma caesia*. *International Journal of Pharmacy and Life Sciences*. 2013;5(4):2664-66.
- Dhanish J, Venugopal A. Medicinal properties of black turmeric: A review. 2018;4(3).
- Choudhary P, Sharma R, Kumar V, et al. Synthesis, characterization and catalytic activity of Bio-MCM-41 for production of bio crude oil via pyrolysis of rice straw. *Waste Biomass Valor*. 2023. <https://doi.org/10.1007/s12649-023-02124-5>
- Sharma N, Rawat R, Lalremruati F, Singh A. Complete plant regeneration of *Valeriana wallichii* DC. on auxin enriched medium and phytochemical analysis. *Plant Sci Today*. 2020;7(4):542-50. <https://doi.org/10.14719/pst.2020.7.4.837>
- Raghav P, Choudhary P, Singh A, et al. Extraction and characterization of oil from seeds of the medicinal plant *Withania coagulans* (Stocks) Dunal (Doda paneer). *Plant Sci Today*. 2022;9(3):698-704. <https://doi.org/10.14719/pst.1649>
- Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology*. 1995;28(1):25-30. [https://doi.org/10.1016/s0023-6438\(95\)80008-5](https://doi.org/10.1016/s0023-6438(95)80008-5)
- Williams LA, O'Connar A, Latore L, Dennis O, Ringer S, Whittaker JA, et al. The *in vitro* anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. *West Indian Med J*. 2008;57(4):327-31.
- Lingegowda DC, Kumar JK, Prasad AGD. FTIR spectroscopic studies on *Cleome gynandra* - comparative analysis of functional group before and after extraction. *Romanian J Biophys*. 2013;22(3-4):137-43.
- Ismail EH, Sabry DY, Mahdy H, Khalil MMH. Synthesis and characterization of some ternary metal complexes of curcumin with 1,10-phenanthroline and their anticancer applications. *Journal Scientific Research*. 2014;6(3):509-19. <http://dx.doi.org/10.3329/jsr.v6i3.18750>
- Pakkirisamy M, Kalakandan SK, Ravichandran K. Phytochemical screening, GC-MS, FT-IR analysis of methanolic extract of *Curcuma caesia* Roxb (black turmeric). *Pharmacognosy Journal*. 2017;9(6):952-56. <https://doi.org/10.5530/pj.2017.6.149>
- Bhattacharya V, M Alagusundaram, Mandal S, Siva R, Karodi R, Jilani AK, et al. Phytochemical analysis of curcumin from the rhizome of *Curcuma longa*. *Tuijin Jishu/Journal of Propulsion Technology*. 2023;44(6):3235-43.
- Varnika, Sharma R, Singh A, Shalini S, Sharma N. Micropropagation and screening of phytochemicals present among *in vitro* raised and wild plants of *Rauvolfia serpentina*. *WJST*. 2020;17(11):1177-93. <https://doi.org/10.48048/wjst.2020.6492>
- Zhang W, Wang Z, Chen T. Curcumol induces apoptosis via caspases independent mitochondrial pathway in human lung adenocarcinoma ASTC-a-1 cells. *Med Oncol*. 2011;28:307-14. <https://doi.org/10.1007/s12032-010-9431-5>
- Ferreira TP, Kuckelhaus IGP, Tschoeke PH, Cangussu AS, Borges JCM, Moura WS, Aguiar RWS. Influence of seasonality on the yield and composition of the essential oil of *Siparuna guianensis* Aublet. *African Journal of Biotechnology*. 2017;16(29):1611-18. <https://doi.org/10.5897/AJB2017.16109>
- Seol GH, Kim KY. Eucalyptol and its role in chronic diseases. In: *Drug Discovery from Mother Nature. Advances in Experimental Medicine and Biology*. Springer, Cham. 2016;929. https://doi.org/10.1007/978-3-319-41342-6_18
- Essien EE, Newby JS, Walker TM, Setzer WN, Ekundayo O. Chemotaxonomic characterization and *in-vitro* antimicrobial and cytotoxic activities of the leaf essential oil of *Curcuma longa* grown in Southern Nigeria. *Medicines (Basel)*. 2015;2(4):340-49. <https://doi.org/10.3390/medicines2040340>
- Makabe H, Maru N, Kuwabara A, Kamo T, Hirota M. Anti-inflammatory sesquiterpenes from *Curcuma zedoaria*. *Nat Prod Res*. 2006;20:680-85. <https://doi.org/10.1080/14786410500462900>
- Kim S, Ko SC, Kim YS, Ha SK, Park HY, Park Y, Lee SH. Determination of *Curcuma longa* L. (Turmeric) Leaf Extraction Conditions Using Response Surface methodology to optimize extraction yield and antioxidant content. *J Food Quality*. 2019; Article ID 7575206. <https://doi.org/10.1155/2019/7575206>
- Arivoli S, Tennyson S, Divya S, Rani S, Marin G. GC-MS analysis of bioactive compounds of *Curcuma longa* Linnaeus (Zingiberaceae) rhizome extract. *J Pharmacogn Phytochem*. 2019;8(6):49-52.
- Anekwe IJ, Chikwendu CI, Amadi ES, Nwogwugwu NU, Ihenetu FC. Gas chromatography-mass spectrometry analysis of bioactive compounds of *Curcuma longa* leaves extract. *Int J Biol Chem Sci*. 2023;17(3):1199-207. <https://doi.org/10.4314/ijbcs.v17i3.34>
- Jain A, Jain P, Bajaj S, Majumdar A, Soni P. Chemoprofiling and antioxidant activity of edible *Curcuma* species. *Food and Humanity*. 2023;1:1027-39. <https://doi.org/10.1016/j.fooHum.2023.08.023>
- Altir NKM, Ali AMA, Gaafar ARZ, Qahtan AA, Abdel-Salam EM, Alshameri A, et al. Phytochemical profile, *in vitro* antioxidant and anti-protein denaturation activities of *Curcuma longa* L. rhizome and leaves. *Open Chemistry*. 2021;19:945-52. <https://doi.org/10.1515/chem-2021-0086>
- Ify OA, Raphael AG, Tochukwu OC, Amarachi OUS, Ikechukwu NA, Madukaihe MJ, et al. The antimicrobial, anti inflammatory and analgesic activities of the rhizome extract of *Curcuma longa* L. (turmeric). *J Advances Bio and Biotech*. 2021;24(6):1-16. <https://doi.org/10.9734/jabb/2021/v24i630217>