Applied Biological Research 26(1): 66-77; (2024)
DOI: 10.48165/abr.2024.26.01.9

ANTI-INFLAMMATORY EFFECT AND GC-MS METABOLOME PROFILING OF *Curcuma haritha* Mangaly & M. Sabu – AN ENDEMIC HERB OF KERALA, SOUTH INDIA

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(Received 13 October, 2023; accepted 15 January, 2024)

ABSTRACT

***Curcuma haritha* (Zingiberaceae) is a less explored endemic ethnomedicinal herb of north Kerala (India). The aim of present study was to evaluate the anti-inflammatory potential and phytochemical profile of methanolic rhizome extract of *C. haritha*. The preliminary assays performed were, the proteinase inhibition and the protein denaturation inhibition tests, followed by cyclooxygenase, lipoxygenase, iNOS and cellular nitrite activity/expression assays in LPS stimulated RAW 264.7 cells. The toxicity of the extract was studied using MTT assay and the expression of COX-2 inflammatory protein in treated cells was determined by indirect ELISA. The phytochemical profiling was done by GC-MS analysis. All the assays revealed a dose-dependent anti-inflammatory effect *in vitro.* The inhibition of inflammatory enzymes, COX-2, Lox and iNOS were observed as 62.16 ± 0.49, 57.25 ± 1.98 and 56.74 ± 2.73%, respectively, at maximum concentration (100 µg mL^{-1}). The cellular nitrite levels and expression of COX-2 mediator protein also decreased in treated cells proving the anti-inflammatory effect of the extract. GC-MS screening revealed the presence of 54 phytoconstituents including anti-inflammatory compounds like curcumenol, chamazulene, etc., Therefore, in the light of above results, *C. haritha* can be considered as a potential anti-inflammatory drug source plant.**

Keywords: *Curcuma haritha*, cyclooxygenase, lipoxygenase, iNOS, cellular nitrite, GC-MS analysis

INTRODUCTION

Curcuma L. is a genus belonging to the family Zingiberaceae, represented by about 80 species within the Indian sub-continent and is largely used in various ethnomedicinal practices (Mangaly and Sabu, 1993). The rhizome of this plant is a rich repository of volatile and non-volatile constituents that imparts significant antiproliferative, antioxidant, anti-inflammatory and antimicrobial properties (Akarchariya *et al*., 2017). Even though most of the species are used as flavouring agents in various cuisines, ornamental plants and as herbal medicine among ethnic groups, only the commercially exploited species like *Curcuma longa* and *C. zedoaria* has gained much scientific attention leaving the other wild species less explored (Dosoky *et al*., 2018). A thorough understanding of the phytochemistry and related bioactivities of such species can be of vital significance as they appear to be a potential source for safer drug lead molecules (Kaliyadasa and Samarasinghe., 2019).

Curcuma haritha Mangaly & M. Sabu., is an endemic species reported from low altitude open fallow lands in various parts of north Kerala (Malabar coast), in the southern Western Ghats (Raj *et*

al., 2008). It is an aromatic herb having yellowish grey rhizomes and a peculiar camphor like smell, seen growing as a mixed population along with *C. aeruginosa* and *C. raktakanta* (Sabu, 2006). Apart from a few phytochemical, antimicrobial and *in vitro* regeneration studies, *C. haritha* is still an underexplored species with respect to its bioactivity potentials (Raj *et al*., 2008).

Inflammatory response is a result of normal immune reactions in the body, but when acute or chronic it can lead to crucial inflammatory diseases (Chen *et al*., 2017). Efficient treatment protocols for inflammatory responses involved the administration of steroidal and non-steroidal anti-inflammatory drugs (NSAIDs), but they have posed much serious contraindications like cardiovascular, renal, hepatic and pulmonary diseases (Bindu *et al*., 2020). This warrants the necessity of discovering safer strategies such as plant-based formulations and compounds to treat inflammatory diseases. Perusal of literature reveals that the anti-inflammatory potential of *C. haritha* has not been reported till date. Therefore, the present study envisions to investigate the potential of *Curcuma haritha* rhizomes in alleviating chronic inflammatory responses *in vitro*, and also delineate the phytochemical diversity to identify possible lead compounds for future *in vivo* and *in silico* anti-inflammatory studies.

MATERIALS AND METHODS

Collection of rhizomes and preparation of extract

The fresh rhizomes of *Curcuma haritha* were collected from various parts of Kozhikode district, North Kerala (India). They were thoroughly cleaned, shade-dried and powdered in a blender. Then 50 g of powdered rhizome sample was extracted using methanol (Carbinol, Hi-LR™, 99% purity) for 16 h in a Soxhlet apparatus to yield a deep reddish colour extract (Redfern *et al*., 2014). Methanol was evaporated completely and the paste stored at 4ºC. From this, the stock extract was prepared in dimethyl sulphoxide (10 mg mL^{-1} DMSO) and used for further analyses.

Preliminary anti-inflammatory studies

The *C. haritha* methanolic extract of 62.5, 125, 250 and 500 µg mL^{-1} concentrations prepared from the stock extract were evaluated for proteinase inhibition activity and protein denaturation inhibition.

Proteinase inhibition activity assay: The reaction mixture was prepared by adding 0.06 mg trypsin, 1 mL 20 Mm Tris HCl buffer (pH 7.4) and 1 mL test sample of different concentrations. These solutions were incubated for 5 min at 37ºC, soon after adding 1mL of 0.8% (w/v) casein. The reaction was terminated after 20 min by adding 2 mL 70% per chloric acid. Then it was centrifuged (@ 3000 rpm for 10 min) and the absorbance was measured (Cary 60 UV-Vis spectrophotometer, Agilent) at 200 nm against buffer as blank (Oyedepo and Femurewa, 1995). The inhibition of proteinase enzyme by *C. haritha* methanolic extract was expressed in percentage values using the formula:

$$\text{Percentage inhibition} = 100 - \left\{\frac{\text{OD of test solution} - \text{OD of product control}}{\text{OD of test control}}\right\} \text{x } 100$$

Protein denaturation inhibition assay: This assay required a test control (0.45 mL BSA (bovine serum albumin) + 0.05 mL distilled water), a test solution (0.45 mL BSA + 0.05 mL sample concentrations) and a product control (0.45 mL distilled water + 0.05 mL sample concentrations). The solutions (pH 6.3) were incubated at 37°C for 20 min and then temperature was increased to 57°C for 3 min. Then 2.5 mL phosphate buffer was added to it after cooling and the absorbance was measured using UV-Visible spectrophotometer (Cary 60, Agilent) at 416 nm (Sakat *et al*., 2010). The inhibition of protein denaturation by *C. haritha* methanolic extract was expressed in percentage values using the formula:

$$\text{Percentage inhibition} = 100 - \left\{\frac{\text{OD of test solution} - \text{OD of product control}}{\text{OD of test control}}\right\} \text{x } 100$$

In vitro anti-inflammatory assays in RAW 264.7 cell lines

LPS stimulated macrophage cell lines RAW 264.7 (procured from NCCS, Pune) were cultured using the standard protocol in antibiotic supplemented DMEM (Dulbecco's modified Eagles medium, Sigma Aldrich, USA) (Babu *et al*., 2020). All the assays were conducted in triplicates and used sample (concentration: 25, 50, and 100 µg mL^{-1} from the stock solution) treated cell lysate while diclofenac sodium (DFS) served as standard drug for comparison. The enzyme assays were expressed as percentage inhibition of activity and the cellular nitrite levels were expressed as µg mL^{-1}.

Cyclooxygenase inhibition: The assay mixture contained 100 µL cell lysate, Tris-HCL buffer and 5 mM hemoglobin incubated for 1 min at 25°C. Then 200 mM arachidonic acid was added and after 20 min the reaction was terminated by adding 0.2 mL of 10% trichloro-acetic acid in HCl and thiobarbituric acid (Walker and Gierse, 2010). The mixture was placed in boiling water bath for 20 min, cooled and centrifuged at 1000 rpm for 5 min. The absorbance of supernatant was measured at 632 nm (Cary 60, Agilent) and the termination of arachidonic acid pathway was measured as function of dose dependant activity of the extract and the values were expressed as percentage of inhibition of cyclooxygenase activity.

Lipoxygenase inhibition: The reaction mixture contained 50 µL cell lysate and 200 µL sodium lineolate in Tris-HCl buffer (pH 7.4) so to make a total volume of 2 mL (Axelrod *et al*., 1981). The enzyme activity was measured from dose dependant increase in absorbance, which directly reflected the formation of 5-hydroxyeicosatetraenoic acid. The absorbance was measured at 234 nm (Cary 60, Agilent) and the activity was expressed as percentage inhibition of lipoxygenase activity.

Inducible nitric oxide synthase activity (iNOS): The cell lysate was homogenised with 2 mL HEPES buffer, and 0.1 mL of this lysate was mixed with 0.1 mL each of L-arginine, manganese chloride, dithiothreitol, NADPH, tetrahydropterin and oxygenated haemoglobin (Salter *et al*., 1996). The absorbance was measured at 401 nm (Cary 60, Agilent) and the dose dependant effect of extract on enzyme activity was expressed as percentage inhibition in enzyme. The percent inhibition in COX-2, Lox and iNOS enzymes was calculated using the formula:

$$\text{Percent inhibition} = \frac{(\text{Absorbance control} - \text{Absorbance test})}{\text{Absorbance control}} \text{X}100$$

Estimation of cellular nitrite levels: The cellular nitrite levels in cell lysate was assayed using Griess reagent test (Lepoivre *et al*., 1990). The absorbance was measured at 540 nm (Cary 60, Agilent) with Griess reagent as blank and diclofenac sodium as standard. The amount of nitrite present in the samples was estimated from standard sodium nitrite curves. The activity was expressed as concentration of cellular nitrite in µg mL^{-1}.

In vitro cytotoxicity assay on RAW 264.7 cells using MTT assay

The cytotoxicity of extract was assessed by MTT assay as per the standard protocol (Riss *et al*., 2013). Two days old monolayer of RAW 264.7 cells were trypsinized and suspended in 10% DMEM. Then 100 µL of this cell suspension was seeded in 96 well plate for 24 h. From sample stock (1 mg mL^{-1} DMEM) five times serially diluted two-fold concentrations in triplicates (100, 50, 25, 12.5, and 6.25 µg in 500 µL DMEM) was added to each well. After 24 h the entire plate was observed using inverted phase contrast microscope (Olympus CKX41 with Optika Pro5 CCD camera) and observations were recorded. The sample was then removed and cells were treated with 30 µL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) for 4 h. The cells were then lysed with 150 µL DMSO. The optical density was measured at 570 nm on a microplate reader (Erba Lisa Scan EM, Germany). The percent inhibition was calculated as:

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{ X } 100$$

The LC_{50} concentration of sample (µg mL^{-1}) was calculated using software in Microsoft excel (ED50 PLUS V.1.0., 2000).

Assay of COX-2 inflammatory mediator using indirect ELISA: The LC_{50} concentration of sample was added to LPS stimulated RAW 264.7 cells, then incubated for 24 h and the supernatant was added to a 96 well plate for overnight incubation at 37°C. Then 200 µL blocking buffer in PBS was added to it and after 1 h, it was washed with PBS and 100 µL primary antibody (COX-2) was added. After 2 h the plates were washed and 100 µL secondary antibody (Horse Radish Peroxidase conjugate, Santacruez, USA) was added to it. The material was incubated for 1 h at room temperature. After washing with PBS tween, 200 µL O-dianizidine hydrochloride was added and incubated for 30 min. The reaction was stopped by using 5N HCl and OD measured at 415 nm using an ELISA reader (Erba Lisa Scan EM, Germany) to determine the concentration of inflammatory mediator protein COX-2. The activity was expressed as µg mL^{-1} using the formula:

$$\text{Activity of antibody} = \frac{\text{OD value}}{\text{Protein concentration}}$$

GC-MS analysis

The volatile components in sample extracts were identified by GC–MS (Shimadzu QP-2010 Plus) with thermal desorption system TD 20. The compounds were identified and tabulated according to their linear retention indices. After comparing the MS fragmentation pattern using the NIST 11 (National Institute of Standards and Technology, US) mass spectra libraries, the individual phytochemical compounds were identified.

Statistical analysis

The data were statistically analysed using IBM SPSS statistics version 20. All the above experiments were conducted in randomised block experimental design and each treatment was replicated 3 times. One way ANOVA followed by Duncan's Multiple Range Test was used to assess the variability and validity of the data collected (Montgomery, 2012). The results were expressed as mean ± standard error. The statistical significance was determined at $p < 0.05$.

RESULTS AND DISCUSSION

Curcuma haritha was initially assessed for its anti-inflammatory potentials by proteinase inhibition and protein denaturation inhibition activities. When considerable activity was observed, the inhibition of major inflammatory enzymes COX-2, Lox and iNOS were also assessed *in vitro* to find a dose dependent effect in the LPS stimulated RAW 264.7 cells.

Protein denaturation and proteinase inhibition assays

The *C. haritha* methanolic extract samples showed a concentration dependant increase in the percentage of inhibition in both the assays. The maximum inhibition observed was 53.25 ± 0.196% in proteinase inhibition activity and 52.17 ± 0.431% in protein denaturation inhibition, both at maximum concentrations (Fig. 1). The proteinase enzymes modulate the chemotactic behaviour of inflammatory cells and activate cellular receptors like PARI (protease activated receptors) causing further inflammatory reactions (Chakraborty and Bhattacharyya, 2013; Modi *et al*., 2019). Therefore, the inhibition of proteinases can be of significant impact in curtailing inflammatory responses. Protein denaturation is another major sign of inflammation in arthritic conditions. It often denotes tissue damage which result in denatured proteins intra- and inter-cellularly (Osman *et al*., 2016). In view of the previous reports and the results of present study, *C. haritha* clearly showed dose dependant inhibition of proteinase activity and protein denaturation activity which is suggestive of its anti-inflammatory efficacy.

In vitro anti-inflammatory assays

The *in vitro* assays showed a steady dose dependant rise in the percent inhibition of cyclooxygenase-2,

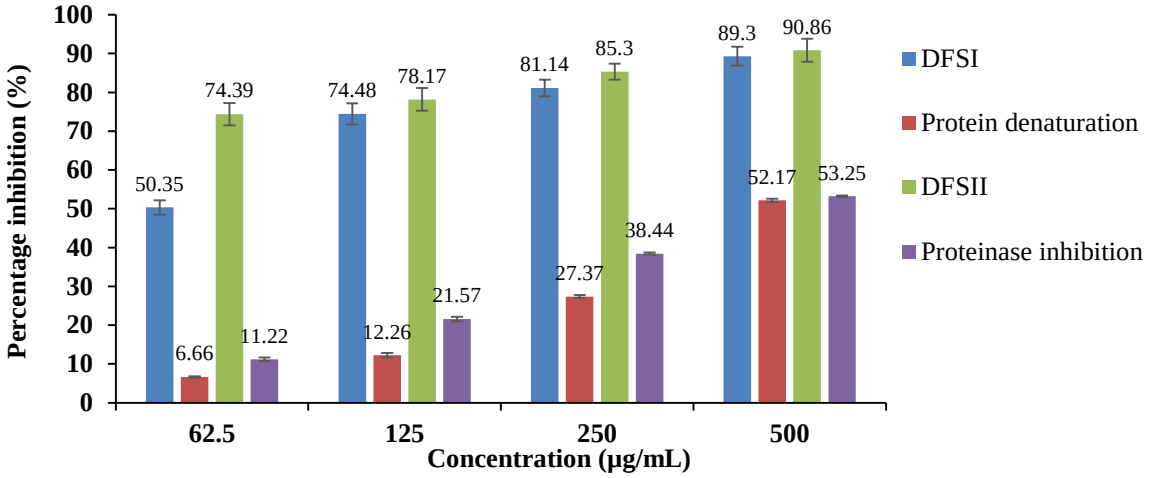

Fig. 1: Protein denaturation activity and proteinase inhibition activities of methanolic extract of *Curcuma haritha* (CHA) and diclofenac sodium (DFS) standard. DFS I – Standard for protein denaturation, DFS II- Standard for proteinase inhibition. Data expressed as mean ± SD of triplicates

lipoxygenase and inducible nitric oxide synthase (Table 1). COX-2 and Lox enzymes are involved in the synthesis of prostaglandins and leukotrienes via arachidonic acid metabolism and, therefore, are often an anti-inflammatory drug target (Kambrath and Thoppil, 2019; Sinha *et al*., 2019). COX-2 is an inflammatory enzyme activated by p38-MAPK pathways and its over- expression can lead to chronic inflammation later causing oncogenesis (Murakami and Ohigashi, 2007). Lipoxygenase (Lox) is another inflammatory gene activated by NF-κB pathway and it exerts pro-inflammatory effects through leukotrienes which is majorly responsible for asthma and rheumatoid arthritis (Sinha *et al*., 2019). The methanolic extract of *C. haritha* showed considerable inhibition in the expression of both these pro-inflammatory enzymes and, therefore, is suggested of a potential anti-inflammatory drug source. Excess nitric oxide (NO) concentration in inflammatory responses is mediated by iNOS activity which converts amino acids arginine and citrulline to NO (El-Gayar *et al*., 2003). The disruption of arginine-iNOS-NO axis during inflammatory vascular injury is the reason for escalated cellular nitrite levels during inflammatory response (Waltz *et al*., 2015). iNOS is a reported as a downstream regulator of inflammatory response that enhances inflammatory response upstream and act as downstream target of NF-κB. The inhibition of iNOS inhibits the acetylation of NF-κB and, therefore, pose anti-inflammatory effects (Nakazawa *et al*., 2017). The dose dependant increase in percent inhibition of iNOS by *C. haritha,* and a subsequent depreciation in the levels of cellular nitrite concentration (Fig. 2) suggest significant anti-inflammatory potential of the extract.

In vitro cytotoxicity assay on RAW 264.7 cells by MTT assay

The *C. haritha* methanolic extract treated cells were observed under an inverted phase contrast microscope to note detectable changes. The morphological changes observed were the rounding and

Table 1: Percent inhibition of major inflammatory enzymes COX-2, Lox and iNOS by *Curcuma haritha* (CHA) methanolic extract and diclofenac sodium (DFS). Data expressed as mean ± SE

Name of the assay	% inhibition (at 100 µg mL^{-1})	Activity of diclofenac sodium (at 100 µg mL^{-1})
Cyclooxygenase activity (%)	62.16 ± 0.492	91.4 ± 2.51
Lipoxygenase activity (%)	57.25 ± 1.98	90.77 ± 2.69
Inducible nitric oxide synthase (%)	56.74 ± 2.73	87.06 ± 3.94

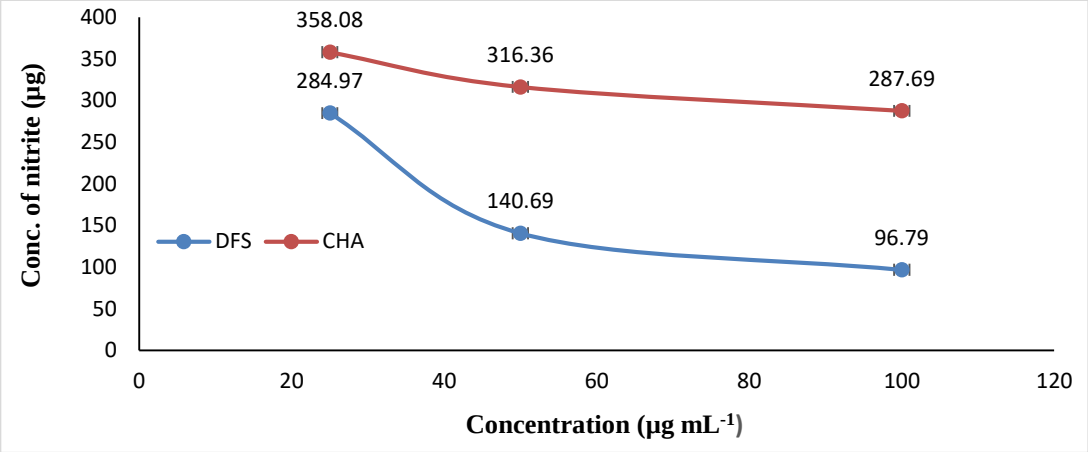

Fig. 2: Cellular nitrite levels (mean ± SE) in *C. haritha* (CHA) treated LPS stimulated RAW 264.7 cells against standard diclofenac sodium (DFS) revealing dose dependant expression

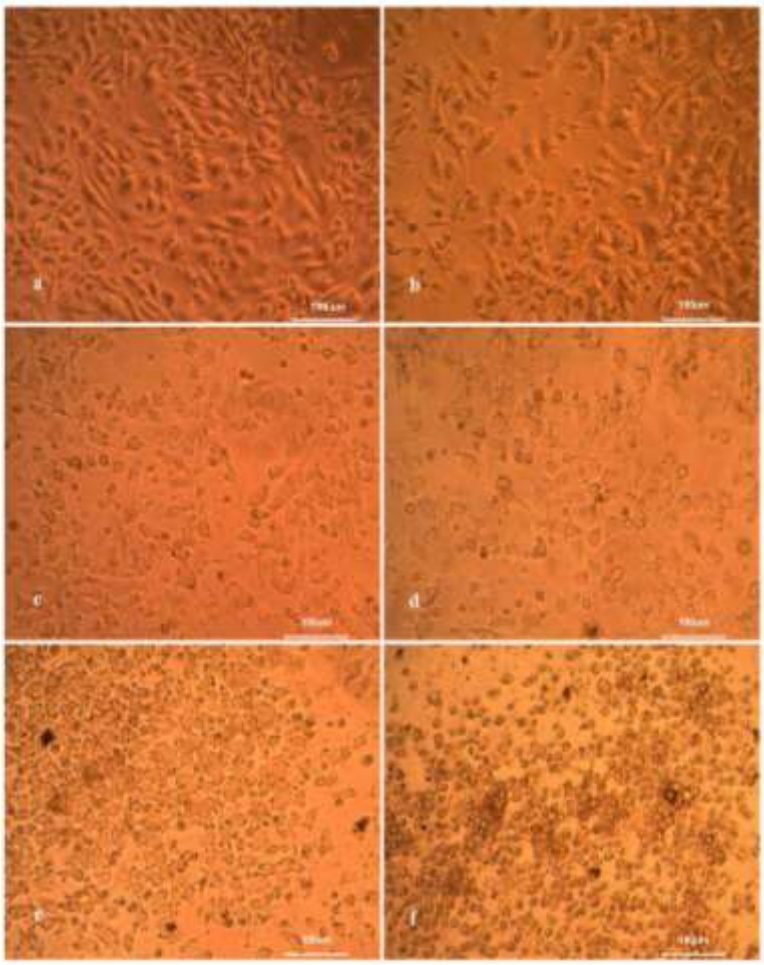

Fig. 3: Microscopic observations on LPS stimulated RAW 264.7 cells after MTT assay at increasing concentrations a. Control, b. 6.25 µg mL^{-1}, c. 12.5 µg mL^{-1}, d. 25 µg mL^{-1}, e. 50 µg mL^{-1} and f. 100 µg mL^{-1} *Curcuma haritha* methanolic extract

shrinking of cells and granulation and vacuolization of cytoplasm which indicated cytotoxicity at higher concentrations (Fig. 3). The spectrophotometric analysis of formazan crystals revealed that the viability of cells gradually decreased with increase in sample concentration. The cell viability at concentrations 6.25, 12.5, 25, 50 and 100 µg mL^{-1} were 91.27 ± 1.55, 74.62 ± 2.83, 66.62 ± 1.23, 63.43 ± 1.25 and 49.32 ± 1.16%, respectively. From the dose dependant effects on the viability of RAW 264.7 cells, it was found that approximately 50% of the treated cells lost their viability at 100 µg mL^{-1}. Therefore, the LC_{50} concentration was 91.32 ± 5.17 µg mL^{-1}. It has been reported that the methanolic crude plant extracts interfere in MTT reaction and tend to show false positive viability percentages (Karakas *et al*., 2017). This possibility is resolved by comparing the calculated percent viability with the microscopic images and both correlated positively.

Assay of COX-2 inflammatory mediator using indirect ELISA

The expression of COX-2 protein was assessed in control and *C. haritha* methanolic extract treated cells and the results are shown in Fig. 4. The COX-2 expression levels were found to be 0.4661 ± 0.00013 and 0.3813 ± 0.00052 activity units mg^{-1} protein in control and treated cells, respectively. COX-2 is the inducible form of cyclooxygenase enzyme and is involved in chronic inflammatory diseases. The suppression in COX-2 level directly decreased the expression of pro-inflammatory mediators in macrophage cell lines (Wang *et al*., 2014). Therefore, the depreciation in COX-2 expression in present study, substantiates the anti-inflammatory effect of *C. haritha*.

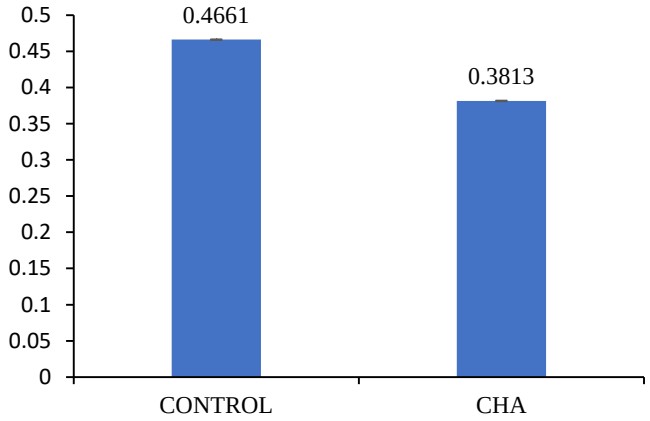

Fig. 4: A quantitative comparison of COX-2 expression in control and treated samples of LPS stimulated RAW 264.7 macrophage cells using indirect ELISA [Data expressed as mean ± SE]

GC-MS analysis

The GC of *C. haritha* methanolic extract revealed 85 peaks (Fig. 5). These peaks (compounds) were further scrutinized and synthetic compounds like phthalates and silanes were omitted for better clarity of results. A total of 54 compounds were identified as per their retention time and peak area percentage (Table 2). The GC-MS profile of essential oil of *C. haritha* rhizome was earlier reported with major compounds like camphor, 1,8-cineol, isoborneol, camphene, linalool, germacrone, etc. (Raj *et al*., 2008). In a growth-related secondary metabolite analysis using different GC-based methods, *C. haritha* essential oil was reported with camphor, germacrane-type compounds and sesquiterpene lactones (Baby *et al*., 2010). The GC-MS profile of methanolic extract of *C. haritha* in present study has also revealed the presence of similar terpenoids like camphor, 1,8 cineol, isofuranodienone, isocurcumenol, curcumenone, spathulenol, etc.

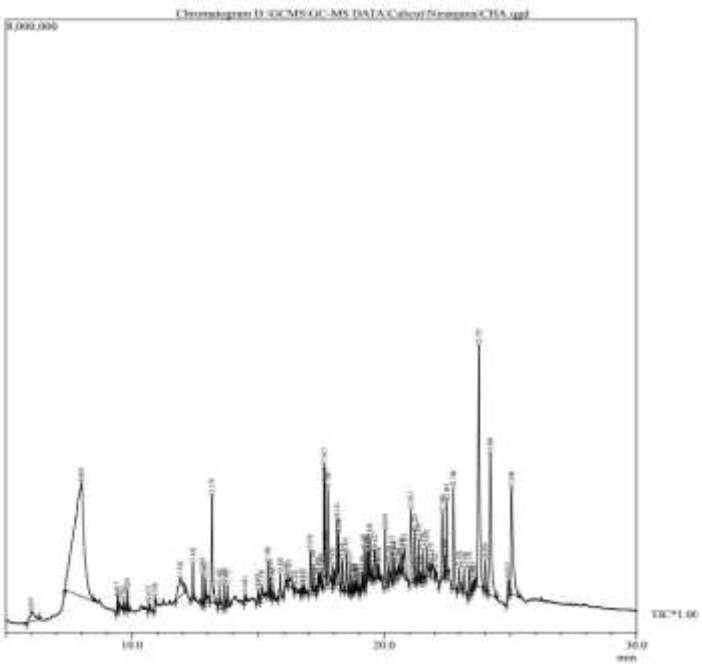

Fig. 5: GC-MS chromatogram of the methanolic extract of *C. haritha* methanolic extract

Out of the 54 compounds obtained in GC-MS profile, 10 compounds were known to possess anti-inflammatory potential from previous reports (Table 3). Curcumenol is a non-toxic sesquiterpenoid with anti-inflammatory effect which acts by inhibiting the NF-KB and MAPK pathways (Yang *et al*., 2021). Chamazulene, another sesquiterpenoid was identified by leukotriene inhibitory action along with antioxidant potentials (Safahyi *et al*., 1994). Zederone is also a sesquiterpene with proven anti-inflammatory action in TPA induced oedema in rat ears (Makabe *et al*., 2006). There is need to

Table 2: Volatile phytocompounds detected in the methanolic rhizome extract of *C. haritha* by GC-MS

S. No.	Retention time (min)	Peak area (%)	Compounds detected
1	9.417	0.26	Camphor
2	9.510	0.19	4H-pyran-4-one,2,3, dihydro-3,5-dihydroxy-6-methyl
3	9.702	0.12	Borneol
4	10.675	0.12	2-acetoxy 1,8-cineole
5	11.916	1.93	Tetritol
6	12.416	0.81	(1R,4R)-10-hydroxycamphor
7	12.803	0.52	Epi-camphor
8	12.911	0.48	3-cyclohexene-1-methanol,5-hydroxy-alpha, alpha,4-trimethyl
9	13.179	3.12	2-butyl-5-methyl-3-(2-methylprop-2-enyl) cyclohexanone
10	13.656	0.47	2-hydroxy-1,1,10, -trimethyl-6,9-epidioxydecalin
11	14.993	0.24	ent-germacra-4(15),5,10(14)-trien-1.beta.-ol
12	15.143	0.38	Gamma-1-cadinene aldehyde
13	15.490	0.30	3- buten-2-ol, 2-methyl-4-(1,3,3-trimethyl-7-oxabicyclo [4.1.0]hept-2-yl)-
14	15.593	0.25	Isofuranodienone
15	15.881	0.64	Isocurcumenol
16	16.102	0.25	Methyl jasmonate
17	16.223	0.63	Methyl alpha-D-galactopyranoside
18	16.476	0.33	Humulene 1,2-epoxide
19	16.737	0.18	Bicyclo[3.3.1]nonan-9-one, 2,4 -dimethyl-3-nitro-(exo)
20	16.833	0.09	Isolongifolene, 9,10-dehydro
21	17.078	1.14	Curcumenol
22	17.185	0.49	Chamazulene
23	17.392	0.14	1-(7-hydroxy-1,6,6-trimethyl-10-oxa-tricyclo[5.2.1.0(2,4)]dec-9-yl)ethanone
24	17.495	0.24	Acorenone
25	17.613	1.91	Isospathulenol
26	17.671	0.77	Cyclohexane,1,1-dimethyl-2,4-bis(1-methylethenyl)-cis-
27	17.784	1.82	(+)-isovelleral
28	18.141	1.27	Ethanone,1,1'-(5-hydroxy-2,2-dimethylbicyclo[4.1.0]heptane-1,7-diyl)bis(1.alpha,5. beta,6.alpha,7.alpha)-
29	18.200	0.52	Curcumenone
30	18.334	0.84	Callitrin
31	18.627	0.50	Platambin
32	18.723	0.24	7-tetracyclo [6.2.1.0(3.8)0(3.9)] undecanol,4,4,11,11 tetramethyl-
33	18.945	0.16	Isoaromadendrene epoxide
34	19.050	0.06	Hinesol
35	19.197	0.58	Spathulenol
36	19.301	0.57	Preg-4-ene-3,20-dione
37	19.358	0.31	Dibutyl phthalate
38	19.627	1.04	Undeca-3,4-diene-2,10-dione,5,6,6-trimethyl
39	20.039	1.27	Viridiflorol
40	20.312	0.58	4-(7,7-dimethylspiro [2.5] octan-8-yl) butan-2-one
41	20.568	0.18	3-buten-2-ol, 2-methyl-4-(1,3,3-trimethyl-7-oxabicyclo [4.1.0] hept-2-yl)-
42	20.689	0.42	Tricyclo[8.6.0E2,9]hexadecan-8,16,KOPF SCHW
43	20.813	1.15	8-methyl-1-tetralone
44	21.053	2.86	Bicyclo[5.1.0]octan-2-one, 4,6-diisopropylidene-8,8-dimethyl-
45	21.356	0.77	7-oxabicyclo [4.1.0] heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-
46	21.692	0.64	Valeranal
47	22.483	1.47	Xanthinin
48	22.748	2.83	Zederone
49	22.974	0.71	4-alpha,10-beta-dihydroxy-1-beta-h,5-alpha-h-guai-11(13)-en-8-alpha,12-olide
50	23.166	0.78	Methyl cedrene
51	23.597	0.15	D-norandrostane-16-ol acetate
52	24.206	6.42	Xanthatin
53	24.917	0.70	Spiro[indoline-3,3'-pyrrolidin]-2-one
54	25.046	5.52	1S,3R,4S,5R,6S-1-hydroxy-2,2,3,4,5,6-hexamethyl-8-oxo-7,9-dioxatricyclo[4.2.1.0 (3,5)] nonane

Table 3: Volatile compounds detected from GC-MS analysis of methanolic extract of *Curcuma haritha* with reported anti-inflammatory property

RT (min)	Compound detected	Bioactivities reported as per literature
11.916	Tetritol	Anti-inflammatory, antioxidant (Babu *et al*., 2013)
16.476	Humulene 1,2-epoxide	Anti-inflammatory (Chaves *et al*., 2008)
16.833	Isolongifolene, 9,10-dehydro	Anti-inflammatory, antibacterial, anticancer (Vasudevan and Neerakkal, 2021)
17.078	Curcumenol	Anti-inflammatory, neuroprotective, anti-tumour (Lo *et al*., 2015)
17.185	Chamazulene	Anti-inflammatory (Jakovlev, 1983)
17.392	1-(7-hydroxy-1,6,6-trimethyl-10-oxa-tricyclo[5.2.1.0(2,4)]dec-9-yl)ethanone	Anti-inflammatory (Ayawa *et al*., 2022)
20.039	Viridiflorol	Anti-inflammatory, antioxidant (Trevizan, 2016)
22.748	Zederone	Anti-inflammatory (Anuchapreeda *et al*., 2018)
24.206	Xanthatin	Anti-inflammatory, apoptosis inducing (Liu *et al*., 2022)
24.917	Spiro[indoline-3,3'-pyrrolidin]-2-one	Anti-inflammatory, anticancer (Kumar and Ritika, 2020)

identify the specific action of these compounds on the concerned inflammatory target proteins *in silico* and its further validation *in vivo*.

Conclusion: *Curcuma* is a repository genus possessing a large number of curcuminoid and non-curcuminoid bioactive compounds that can act as potential sources of many new age drug formulations against a multitude of diseases. Inflammation is a pathophysiological condition often treated with harmful steroidal and non-steroidal formulations. In this scenario, phytochemicals are safer substitutes with chemo-preventive properties, reasonable modes of action and low levels of toxicity. In this study *Curcuma haritha*, an endemic species of Kerala, was evaluated for its *in vitro* anti-inflammatory potential. Preliminary biochemical assays revealed considerable activity and it was reaffirmed by various assays like COX-2, Lox and iNOS inhibitory assays in LPS stimulated RAW 264.7 cells. The GC-MS screening of methanolic extract of *C. haritha* revealed the presence of compounds, largely terpenoids, with reported anti-inflammatory activities. Therefore, the species *C. haritha* can be used as a potential source of lead molecules in anti-inflammatory drug research.

Acknowledgement: The authors are thankful to the Central Sophisticated Instrumentation Facility (CSIF), University of Calicut, Kerala (India) for the provision of technical support during this study.

Conflict of interest: The authors declare that there are no conflicts of interest related to this study.

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