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RESEARCH ARTICLES

Evaluation of COX-2 production, anticancer efficacy against MCF-7 cell lines, and antioxidant activity of *Garcinia conicarpa*, a novel endemic species of the Western Ghats

M. V. Divyalakshmi[1] · J. E. Thoppil[1]

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Abstract
Garcinia species are members of the Cluciaceae family and are rich in secondary metabolites. In the Western Ghats, *Garcinia conicarpa* is a recently discovered endemic species. In this study, the methanolic leaf extract of *Garcinia conicarpa* (GC) is examined for its anti-inflammatory, anti-cancer, and antioxidant properties. Significant phytocompounds were discovered using HR LC–MS. To investigate the antioxidant capacity of extracts, four different radical scavenging assays, such as the hydroxyl radical scavenging assay, the nitric oxide radical scavenging assay, the ABTS radical scavenging assay, and the DPPH radical scavenging assay, were used. Through assays such as inhibition of protein denaturation, proteinase inhibitory activity, cycloxygenase (COX) activity, lipoxygenase (LOX) activity, and inducible nitric oxide synthase activity assays, *G. conicarpa*'s anti-inflammatory properties were assessed. While anti-proliferative activity was studied using breast cancer MCF-7 cell lines. The activity of important compounds isolated through HR LC–MS was analysed. This is the first time the antioxidant activity of the methanolic leaf extract of *G. conicarpa* has been investigated. Leaf extract shows high to medium antioxidant activity in all the assays. *G. conicarpa* also has anti-inflammatory activity in a dose-dependent manner in all the biochemical assays, and the extract reduced the cyclooxygenase enzyme (COX-2) level from 0.84 to 0.75 U/mL. In the anti-cancerous study, the cell viability percentage of MCF-7 cell lines ranged from 95.7% to 71.8%. So, it is evident from this study that *G. conicarpa* methanolic leaf extract is a unique natural anti-inflammatory and antioxidant treasure with the capability to combat breast cancer.

Keywords *Garcinia conicarpa* · HR LC–MS · Antioxidant activity · Anti-inflammatory · Anticancer

Abbreviations

GC	*Garcinia conicarpa*
ROS	Reactive oxygen species
HR-LC MS	High resolution-liquid chromatography mass spectroscopy
ABTS	2, 2-Azinobis-3-ethylbenzothiazoline-6-sulphonate
DPPH	2, 2-Diphenyl-2-picrylhydrazyl
COX	Cyclooxygenase
LOX	Lipoxygenase
iNOS	Inducible nitric oxide synthase
HEPES	4-(2-Hydroxyethyl)-piperazineethanesulfonic acid
LPS	Lipopolysaccharide

✉ M. V. Divyalakshmi
mvdivyalakshmi@gmail.com

J. E. Thoppil
jethoppil@gmail.com

[1] Cell and Molecular Biology Division, Department of Botany, University of Calicut, Kerala 673635, India

Introduction

Plants have been used as therapeutics for a very long time. *Garcinia* can be found in tropical Africa, New Caledonia, Polynesia, Brazil, and Asia. *Garcinia*, the largest genus belonging to the family Clusiaceae, is one of the least explored groups of plants in the Western Ghats. *Garcinia* has been utilized in several industrial sectors. In recent years, the variety and complexity of plant metabolites have been explored for pharmaceutical purposes. *Garcinia* is rich in phytocompounds such as xanthones, polyphenols, and bioflavonoids. Benzophenones such as garcinol, 7-epiclusianone, flavonoids like procyanidine, and the biflavonoid fukugentin have been isolated from the leaves of different

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Garcinia species. The *Garcinia* genus has been widely used for the treatment of inflammation, microbial infection, cancer, and obesity. It is also attributed to the following bioactive potentials: antioxidant, antitumoral, antifungal, anticancer, antimicrobial and antiviral, (Espirito santo et al. 2020). Rising pollution, food toxicity, and other factors in the modern world have an impact on the formation of ROS, which damages DNA and causes the emergence of a range of chronic and degenerative ailments. During normal aerobic cellular metabolism, different free radicals are generated. An imbalance in ROS production and antioxidant defences results in oxidative stress. Excessive reactive oxygen species (ROS) formation disrupts normal physiological processes. Antioxidants can scavenge free radicals and prevent several chronic and degenerative diseases. They have activities such as anti-ageing, anti-inflammatory, anti-atherosclerosis, and anti-cancer. ROS can trigger signalling pathways for inflammation. All higher vertebrates use inflammation as a crucial survival strategy in response to infection and damage (Liu et al. 2017). Different proinflammatory mediators will be activated by the action of ROS. ROS imbalance leads to inflammation, which, together with oxidative stress, contributes to diseases such as cancer (Yeshi et al. 2022). One of the reasons for the high mortality rate among women worldwide is breast cancer. In modern science, the role of inflammation and the immune system during the treatment of cancer has been deeply studied. Inflammation and cancer are closely related, and several anti-cancer agents can be used for the treatment of inflammation (Rayburn et al. 2009). Metastasizing cancer cells to distant organs makes it more severe (Tang et al. 2013). Natural products derived from plants have fewer side effects than synthetic chemotherapeutic drugs. *Garcinia conicarpa*, with ovoid-conical fruits, ovate or rarely oblong leaves, and yellow exudates, was previously considered a variety of *Garcinia gummi-gutta* and reinstated as a separate species in 2021 (Shameer et al. 2021). Plant-based antioxidants, anticancer agents, and anti-inflammatory compounds have enormous benefits for human consumption and as dietary supplements. A better understanding of the antioxidant, anti-inflammatory, and anti-cancer activities of *Garcinia conicarpa* helps in the development of effective drugs. In this work, phytocompounds in *G. conicarpa* have antioxidant, anti-inflammatory, and anticancer activities. Through this work, phytochemical constituents, antioxidant, anti-inflammatory, and anticancerous activities of *G. conicarpa* were studied. This is a novel work on the bioactivities of methanolic leaf extracts of *Garcinia conicarpa* leaves.

Materials and methods

Collection and identification

Healthy leaves of *Garcinia conicarpa* Wight were collected from Thollayiramkandi, Wayanad district, Kerala, and authenticated. The habit of *Garcinia conicarpa* is shown in Fig. 1. Healthy leaves were washed, shade-dried, and powdered using an electric blender. Three solvents, such as methanol, ethanol, and DMSO, were selected for the preliminary qualitative detection of various phytochemicals. More compounds were eluted in methanol, and hence methanol was chosen for further study. Powdered leaf was subjected to soxhlet extraction with methanol as solvent. The extract was cooled and filtered, and methanol was completely evaporated. The dried, concentrated extract was kept at 40 °C in an airtight glass vial for further experiments.

Phytochemical screening

High resolution-liquid chromatography mass spectroscopy (HR-LC MS)

Nonvolatile phytocompounds were analysed using HR LC–MS Q-TOF (Agilent g6550A, USA). HiP sampler (model G4226A) with ejection speed of 100 µL/min, flush

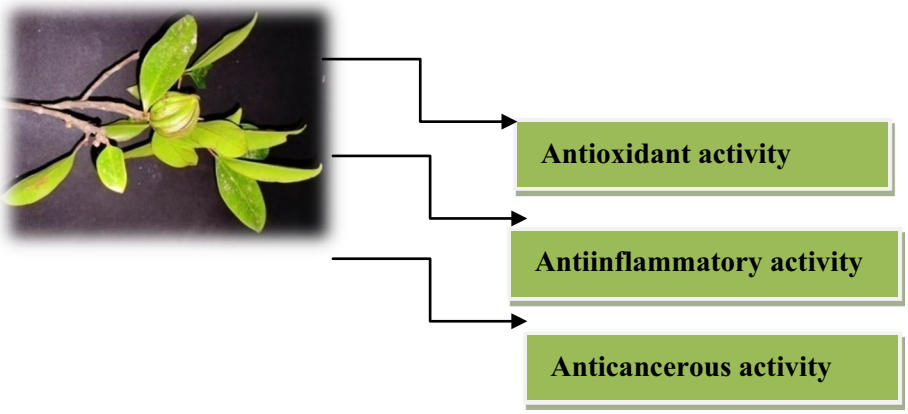


Fig. 1 Habit and activities of *Garcinia conicarpa*

out factor of 5 µL and 8 µL injection volumes, binary gradient solvent pump, column compartment, and quadrupole time of flight mass spectrometer (MS Q-TOF) with a dual Agilent Jet Stream Electrospray (AJS ES) ion source that jointly work in the liquid chromatographic system. 95% water and 5% acetonitrile at a flow rate of 0.5 mL/min were used as the mobile phase for elution.

In vitro antioxidant activities

In-vitro DPPH, ABTS, nitric oxide and hydroxyl radical scavenging assays were done for the determination of antioxidant activities.

DPPH radical scavenging assay

The approach reported by Chang et al. (2001) was slightly modified to employ the stable radical 2, 2-diphenyl-2-picrylhydrazyl (DPPH) to assess the potential for natural antioxidants. DPPH was combined with various sample concentrations and left at room temperature for 20 min in the dark. Ascorbic acid was used as a reference to evaluate absorbance at 516 nm. Inhibitory concentration 50 (IC_{50}), the concentration necessary to capture 50% of DPPH, was used to indicate antioxidant activity.

ABTS radical-scavenging activity

The method outlined by Re et al. (1999) was used to assess the 2, 2-azinobis-3-ethylbenzothiazoline-6-sulphonate (ABTS) radical cation scavenging activity of sample G. conicarpa. 50 mL of 20 mM ABTS and 0.3 mL of 17 mM potassium persulfate were combined to create the ABTS solution. To the 0.2 mL of different concentrations of sample, 1 mL of distilled water and 0.16 mL of prepared ABTS solution was added. A 20-min incubation period was followed by a 734 nm absorbance measurement. The standard utilised was ascorbic acid.

Nitric oxide radical scavenging assay

The Kumaran and Karunakaran (2006) method was slightly modified to measure the nitric oxide radical scavenging capacity. Sodium nitroprusside (5 $mmolL^{-1}$) in phosphate buffered saline (pH 7.4) combined with different concentrations of the sample was used. After 30 min of incubation at 25 °C, stable nitrite and nitrates produced from unstable nitric oxide were detected through the Greiss reaction. Absorbance measured at 546 nm with gallic acid was used as the reference.

Griess reagent: 1% sulphanilamide, 2% phosphoric acid, and 0.1% N-1-naphthyl ethylene diaminedihydrochloride.

Hydroxyl radical scavenging assay

A hydroxyl radical scavenging assay was carried out using a method that was slightly modified from the method of Kunchandy and Rao (1990). A 500 µl reaction mixture [2 deoxy-2 ribose (2.8 mM), $FeCl_3$ (100 µm), EDTA (100 µm), H_2O_2 (1.0 mM), ascorbic acid (100 µm) in KH_2PO_4-KOH buffer (20 mM, pH 7.4)] was diluted to a final volume of 1 mL and added to the sample at various concentrations. After one hour of incubation at 37 °C, 1 mL of 2.8% TCA and 1 mL of 1% aqueous TBA were added. After 15 min at 90 °C of incubation, the absorbance was measured at 532 nm. Gallic acid served as a point of comparison.

Using the following formula, scavenging activities were calculated:

$$\text{Percentage of inhibition} = \frac{\text{control} - \text{test}}{\text{control}} \times 100$$

In vitro anti-inflammatory activity

Using in vitro biochemical assays, anti-inflammatory potential of the extract was analysed.

Inhibition of protein denaturation

The ability of the leaf's methanolic extract to prevent protein denaturation was assessed using the approach of Mizushima and Kobayashi (1968). The test solution contained various sample concentrations along with 0.45 mL of bovine serum albumin. The reference solution was diclofenac sodium. Samples were incubated for 20 min at 37 ℃ and an acidic pH of 6.3. Later, for 3 min, the temperature was raised to 57 ℃. After cooling and adding 2.5 mL of phosphate buffer, the absorbance was measured at 416 nm.

Proteinase inhibitory activity

The extract's proteinase inhibitory activity was examined using the approach of Oyedepo and Femurewa (1995). After being incubated at 37 °C for 5 min, a 1 mL test sample of various concentrations was combined with a 2 mL reaction mixture consisting of 0.06 mg of trypsin, 1 mL of 20 mM Tris HCl buffer (pH 7.4), and 1 mL of 0.8% (w/v) casein. For ceasing the reaction after 20 min, 2 mL of 70% perchloric acid was added. Centrifuging the hazy suspension at 3000 rpm for 10 min allowed for the measurement of absorbance at 200 nm using buffer as a blank.

$$\% \text{ inhibition} = 100 - \frac{(\text{OD of test solution} - \text{OD of product control}) \times 100}{\text{OD of test control}}$$

Anti-inflammatory activity on raw cell INE

DMEM (Sigma Aldrich, USA) was used to maintain the RAW 264.7 (macrophage) cell line. In the 25 cm2 tissue culture flask, the cells were activated with 1 µL of lipopolysaccharide (LPS: 1 g/mL) once they reached 60% confluency. The LPS-stimulated raw cells were exposed to various doses of the samples, and after 24 h the cell lysate was used to perform the anti-inflammatory assays. Culturing of cell lines were mentioned in 4.1.

Cyclooxygenase activity

The impact of plant extract on COX activity was evaluated using the Walker and Gierse (2010) methodology. Glutathione (5 mM/L), haemoglobin (5 mM/L), and Tris–HCl buffer (pH 8) were added to 100 µL of cell lysate and left to sit at 25 °C for one minute. The reaction was initiated by the addition of arachidonic acid 200 mM/L and terminated after 20 min incubation at 37 °C, by the addition 200 µL of 10% trichloroacetic acid in 1 N hydrochloric acid. The centrifugal separation was combined with 200 µL of 1% thiobarbiturate, and the tubes were then heated for 20 min. After 3 more minutes of centrifugation, absorbance was measured at 632 nm.

Lipoxygenase activity

The determination of LOX activity was done as per Axelrod et al. (1981). Tris–HCl buffer (pH 7.4), 50 µL of cell lysate, and 200 µL sodium linoleate together form the reaction mixture. The LOX activity was monitored as an increase in absorbance at 234 nm, which reflects the formation of 5-hydroxyeicosatetraenoic acid. Diclofenac was used as the control for both cyclooxygenase and lipoxygenase activity and the percentage of inhibition was calculated as follows.

$$\text{Percentage of inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test}) \times 100}{\text{Absorbance of control}}$$

Inducible nitric oxide synthase activity

Using the method described by Salter et al. (1996), inducible nitric oxide synthase activity was determined. Using 2 mL of 4-(2-hydroxyethyl)-piperazineethanesulfonic acid (HEPES) buffer, the cell lysate was homogenised. 0.1 mL of the cell lysate was then mixed with L-arginine, manganese chloride dithiothreitol (DTT), NADPH, tetrahydropterin, and oxygenated haemoglobin at the appropriate amount in the protocol. Absorbance was measured at 401 nm.

Cellular nitrate level

According to the method of Lepoivre et al. (1990), to 0.5 mL of cell lysate, 0.1 mL of 3% sulphosalicylic acid was added and vortexed well for 30 min. Later samples were centrifuged at 5000 rpm for 15 min. 30 µl of 10% NaOH was added, followed by 300 µl of Tris–HCl buffer, which was added to the supernatant. Absorbance was read at 540 nm after addition of 530 µL Griess reagent and incubation in the dark for 10–15 min. A sodium nitrite solution was used as the standard.

ELISA: To estimate inflammatory mediators

The LC_{50} concentration of sample was added to LPS-stimulated cells, and the cells were then incubated for 24 h at 37 °C with humidified 5% CO_2. The LPS-induced cells were taken as controls without the sample. 100 µl of supernatant was added to the 96-well plate after incubation and left there for an overnight period at 37 °C. Following a PBS-based wash, 200 µl of freshly produced blocking buffer was added and left at room temperature for one hour. At room temperature, 100 µl of primary antibodies (COX) were added and left for 2 h. After incubation, it underwent two further PBS washes. 100 µL of secondary antibody (HRP conjugate, Santacruez, USA) was added and left for 1 h at room temperature. After washing with PBS TWEEN, 200 µL of O-dianizdine hydrochloride [1 mg/100 mL methanol + 21 mL citrate buffer (pH 5) + 60 mL H_2O_2: (Sigma Aldrich,USA)] was added and left for 30 min at room temperature. The reaction was terminated by adding 50 µl 5N HCL. OD was read at 415 nm in an ELISA reader.

Activity of antibody = OD Value/ Protein concentration.

Anticancerous activity

Culturing of cell lines

MCF-7 (human breast cancer) cell lines, L929 cells, and RAW cell lines (For anti-inflammatory studies).

L929 cells, RAW cells, and MCF-7 (human breast cancer) cell lines were purchased from NCCS in Pune, India. Cell lines were maintained in Dulbecco's Modified Eagle Medium (Sigma Aldrich, USA) supplemented with 10%

FBS, L-glutamine, sodium bicarbonate, and an antibiotic solution containing: penicillin (100 U/mL), streptomycin (100 µg/mL), and amphotericin B (2.5 µg/m). In 96-well tissue culture plates, 100 µL of cell suspension was seeded, and the plates were then cultured at 37 °C in a humidified 5% CO_2 incubator. After draining the growing media, 100 µL (1 mg of plant extract diluted in 1 mL DMEM) were applied in triplicate to each well and incubated at 37 ℃ in a humidified 5% CO2 incubator. Untreated cells were kept around as controls. Using an inverted phase contrast tissue culture microscope (Olympus CKX41 with an Optika Pro5 CCD camera), the entire plate was examined after 24 h treatment. Following a 24-h incubation period, the sample content in the wells was removed, and both the test and control wells supplied 30 µL of MTT solution (15 mg of MTT (Sigma, M-5655) diluted in 3 mL PBS). 100 µL of MTT solubilization solution (dimethyl sulphoxide, DMSO, Sigma Aldrich, USA) was added to dissolve the formazan crystals after the incubation at 37 °C in a CO_2 incubator for 4 h.

A wavelength of 540 nm was used to measure the absorbance.

The percentage viability was calculated using the formula,

$$\% \text{ of viability } = \frac{\text{Mean OD of samples } * 100}{\text{Mean OD of control group}}$$

Statistical analysis

Statistical comparisons of anti-oxidant, anti-inflammatory and anti-cancerous data were calculated using ED50 PLUS V1.0 software. Data obtained were subjected to one-way analysis of variance (ANOVA), followed by DMRT (Duncan's multiple range test). Each conducted in triplicates and results were expressed as mean ± standard error (SE).

Results

Phytochemical analysis

In addition to having nutrients like carbohydrates, proteins, vitamins, and minerals, plants also have an abundance of secondary metabolites including phenols and flavonoids that significantly contribute to their therapeutic properties. Different metabolites isolated from the genus *Garcinia* have properties such as anticancerous, as free-radicalscavenging, antiulcer, anti-inflammatory etc. (Espirito Santo et al. 2020).

Through HR LC–MS analysis different classes of phytocompounds were evaluated. Table 1 depicts important compounds and biological properties such as antioxidant, anti-inflammatory and anticancer. All of these discoveries highlighted the diversity of secondary metabolites and the necessity for future investigations into their potential biological functions. The liquid chromatogram of *Garcinia conicarpa* was shown in Fig. 2. Here both positive and negative ionisation modes are used for the analysis of compounds.

Free radical scavenging assay

Reactive oxygen species were generated by the reaction between free radicals and oxygen within the cells. Diminished antioxidant activity can lead to different pathological conditions. Through a variety of mechanisms, antioxidants have an inhibitory influence on oxidation processes. Using a variety of assays, antioxidant activity can be determined (Shahidi and Zhong 2015). DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity is an easy way to determine the scavenging activity of antioxidants. The results were expressed in the inhibition percentage of radicals in Fig. 3c. For *G. conicarpa*, an inhibition percentage of 75.77 ± 0.34 was observed at higher concentrations (200 µg/

Table 1 Previous reports of phytocompounds revealed through HR LC–MS analysis with their bioactivities (Divyalakshmi and Thoppil (2023))

Sl.No	Compound	Molecular mass	m/z	Class	Activities	References
1	Zapotin	342.1	365.1	Flavanoid	Antiinflammatory, Antioxidant, Anticancer	Strawa et al. 2021
2	7- Dehydrologanin tetraacetate	556.1	579.1	Terpene glycoside	Antiinflammatory, Antioxidant	Wong et al. 2022
3	1-O-Sinapoylglucose	386.1	409.1	Phenolic acid	Antioxidant	Zhang et al. 2020
4	Nigakilactone B	392.4	415.2	Organic compound	Antiinflammatory, Antioxidant, Anticancer	Mohd et al., 2020
5	Somniferine	608.2	609.2	Morphinane alkaloid	Antiinflammatory Antioxidant, Anticancer	Kalra et al., 2017
6	Pubesenolide	458.2977	459.3048	Phytosteroid	Anticancer	Santagata et al. 2012
7	Euphornin	584.2952	607.2845	Diterpene	Anticancer	Jannet et al. 2017

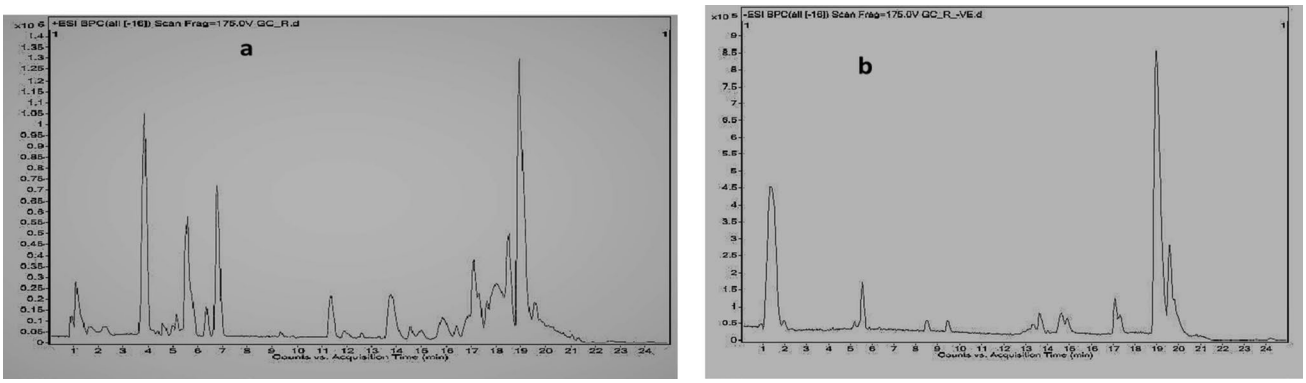


Fig. 2 Liquid chromatogram of *G. conicarpa* (**a**) Positive ionisation mode; (**b**) Negative ionisation mode

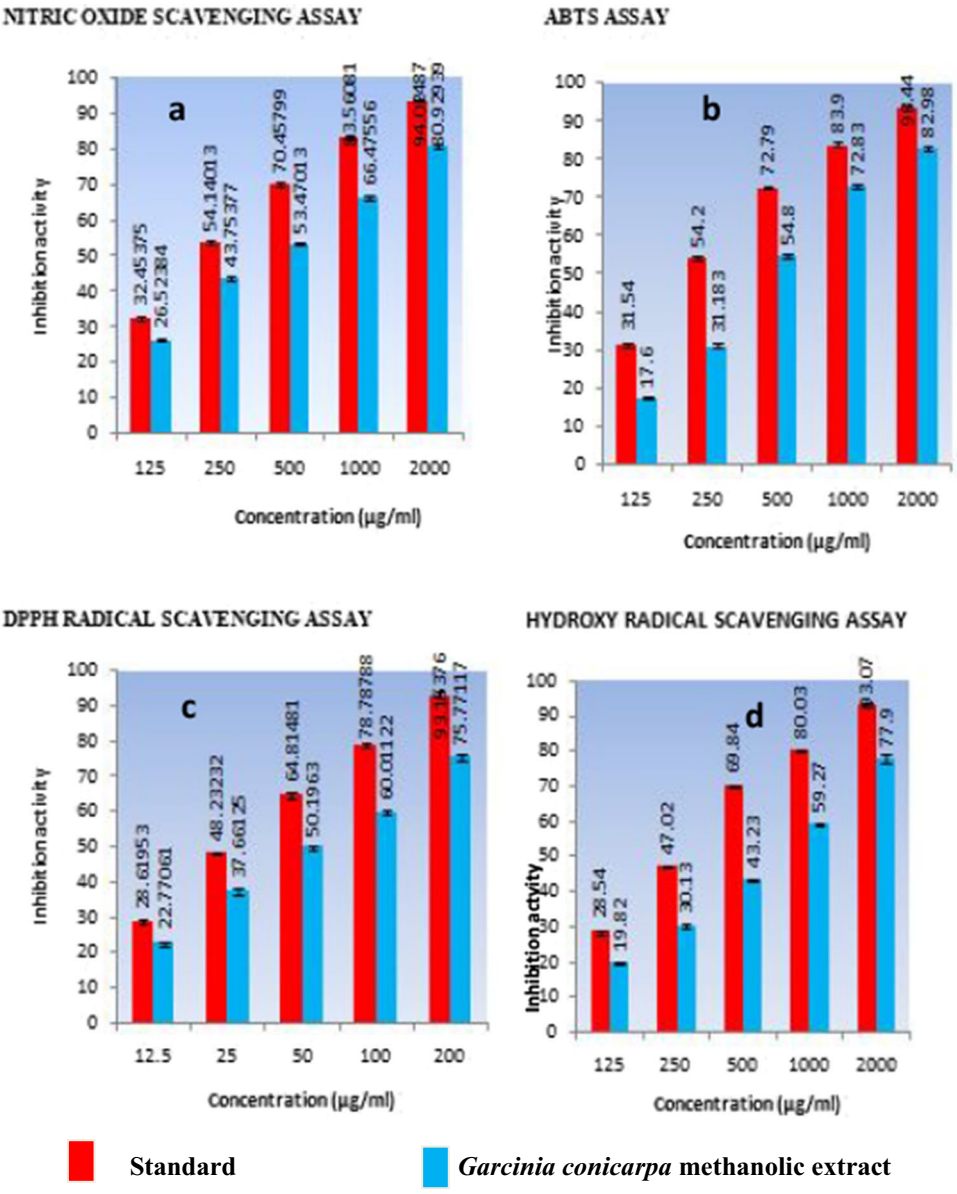


Fig. 3 (**a**) Nitric oxide free radical scavenging assay (**b**) ABTS free radical scsvenging assay (**c**) DPPH free radical scavenging assay (**d**) Hydroxyl free radical scavenging assay

mL), with an IC_{50} value of 47.955 µg/mL. Ascorbic acid was used as the reference, with an inhibition percentage of 93.15 ± 0.72 and an IC_{50} value of 32.179 µg/mL. Increasing scavenging activity was confirmed by decreasing the intensity of the purple color. Using a hydroxyl radical scavenging assay, the scavenging of OH^- was estimated. Hydroxyl radicals can cause lipid peroxidation, protein degradation, and membrane disruption, which can cause significant harm to organisms as a whole. Here, gallic acid was used as the standard (IC_{50} 305.93 ± 0.58). *G. conicarpa* has the highest percentage of inhibition at a concentration of 2000 µg/mL with an IC_{50} value of 743.52 µg/mL shown in Fig. 3d. On the other hand, in the nitric oxide radical scavenging assay (Fig. 3a), there is a dose-dependent increase in scavenging activity. Nitric oxide is an unstable radical; its scavenging ability was determined through Griess reaction. The reaction between NO and the superoxide anion radical results in the formation of peroxynitrite, which causes damage by interacting with lipids, DNA, and proteins (Marković et al. 2017). On a comparative basis, *G. conicarpa* has better activity in quenching nitric oxide, with an IC_{50} value of 421.995 µg/mL. Using standard procedures for the evaluation of the antioxidant potential of the plant extracts, an ABTS assay was also conducted (Fig. 3b). Table 2 shows the comparative IC_{50} values of standard and *G. conicarpa*.

Hydrogen-donating antioxidants present in the extract reduce the ABTS + + radical cation into ABTS. Ascorbic acid with an IC_{50} 226.83 ± 0.69 was used as the reference. *G. conicarpa* shows an inhibition percentage of 82.98% at the higher concentration, with an IC_{50} value of 448.70 µg/mL. In all the antioxidant assays, there is a dose-dependent percentage of inhibition, but the IC_{50} value of *G. conicarpa* has a value nearer to the standard that was only seen in the DPPH and nitric oxide scavenging assays. This is the first study to investigate the antioxidant activity of *Garcinia conicarpa* methanolic leaf extract.

Anti-inflammatory activity

One of the well-documented causes of inflammation is protein denaturation (Kiranmayi et al. 2018). Hence, the ability to reduce protein denaturation indicates higher anti-inflammatory efficacy. Proteinase inhibitors provide protection against different proteinases of leukocytes during inflammatory processes (Gunathilake et al. 2018). The percentage of inhibition increases with an increase in concentration in both the protein inhibition assay and the protein denaturation assay. In the protein inhibition assay (Fig. 4a), the percentage of inhibition at higher concentrations (500 µg/mL) was found to be 85.17 ± 0.65%, with an IC_{50} of 224.60 ± 0.64 µg/mL while in protein denaturation (Fig. 4b), an inhibition percentage of 81.48 ± 0.48% was observed, with an IC_{50} of 177.67 ± 0.61 µg/mL. Diclofenac sodium was used as the standard. The percentage of cell viability on RAW 264.7 cells at different concentrations of *G. conicarpa* was evaluated, and the LC_{50} of the extract was found to be 170.00348 µg/mL through the MTT assay in which cells show only lesser toxic.

Table 2 IC_{50} values of Standard and *Garcinia conicarpa*

Assays	Standard	IC_{50} value of standard (µg/mL) ± SE	IC_{50} value of *G. conicarpa* (µg/mL) ± SE
ABTS assay	Ascorbic acid	226.83 ± 0.69	448.70 ± 1.27
DPPH radical scavenging assay	Ascorbic acid	32.179 ± 0.32	47.95 ± .61
Hydroxyl radical scavenging assay	Gallic acid	305.93 ± 0.58	743.52 ± .74
Nitric oxide radical scavenging assay	Gallic acid	226.136 ± 0.67	421.99 ± .59

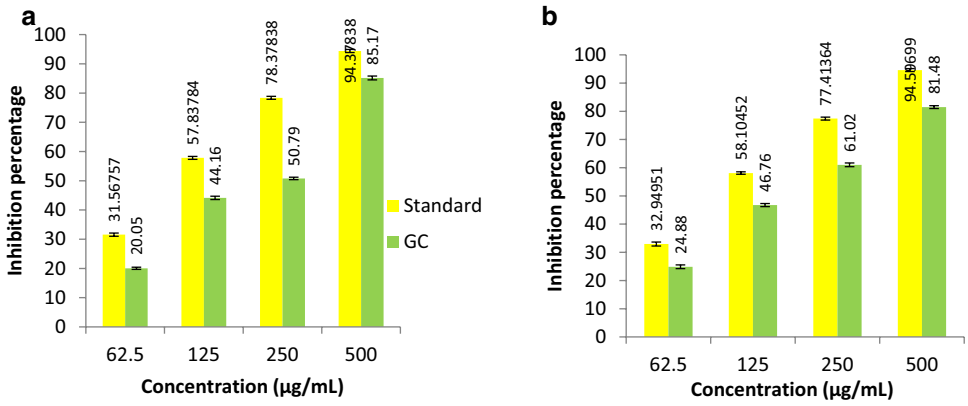
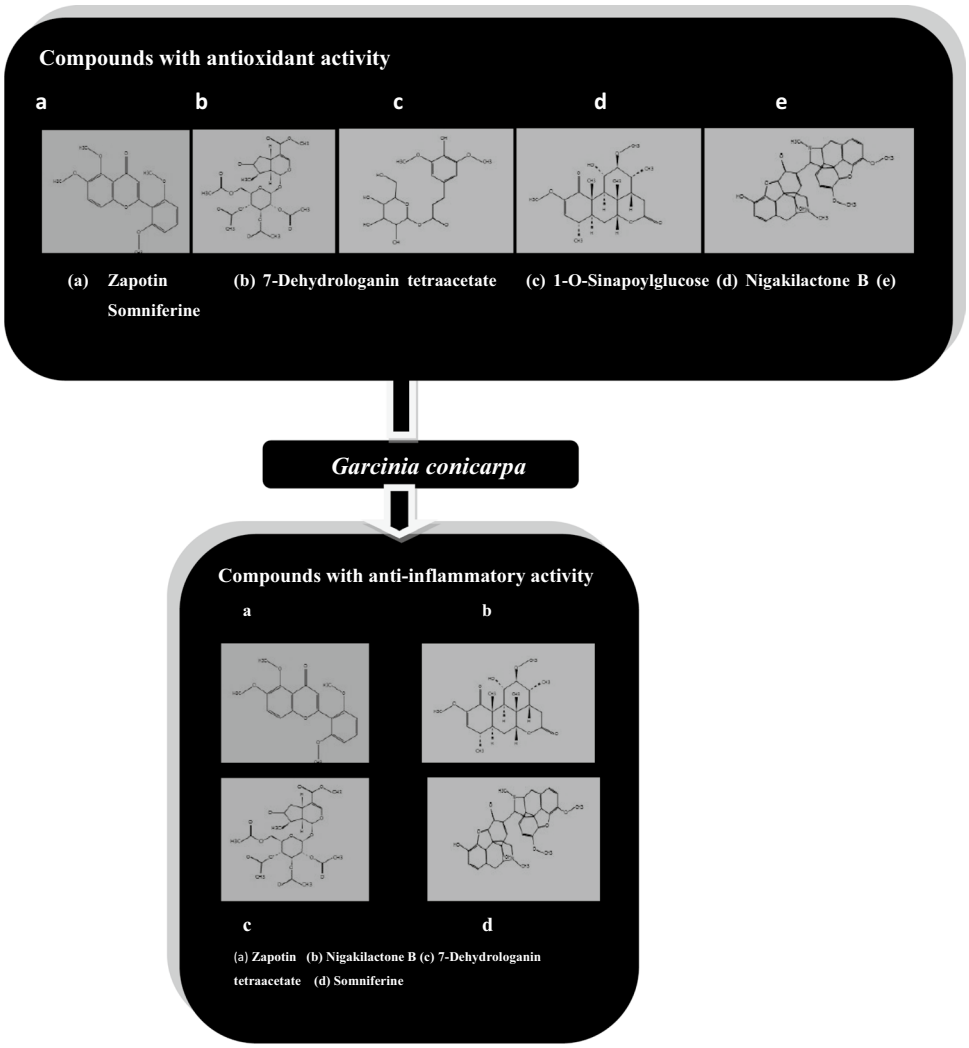


Fig. 4 **a** Proteinase inhibitory activity **b** Inhibition of protein denaturation



Several inflammatory enzymes are activated and released during inflammatory responses. Different mediators in a variety of inflammatory events are formed by the catalytic action of lipoxygenase (LOX) on polyunsaturated fatty acids. A strong LOX inhibitor can act as an effective anti-inflammatory agent. NO is produced from L-arginine by the inducible NO synthase (iNOS) during inflammation, activating the cyclooxygenase enzymes 1 and 2. COX-1 is present in most cells, while COX-2 is activated by inflammation and proinflammatory cytokines. Additionally, the inhibition of the lipoxygenase enzyme and nitric oxide radical scavenging demonstrate anti-inflammatory efficacy. When the efficacy of methanolic leaf extract on the reduction of cycloxygenase and lipoxygenase enzymes on LPS stimulated RAW 264.7 cells [Fig. 5 (a) and (b)], were evaluated there was an increase in the percentage of inhibition from 14.3 ± 0.49 to 50.8 ± 0.45 in COX activity and 44.8 ± 0.48 to 74.8 ± 0.49 for LOX activity with the increasing concentration. LC_{50} values of COX and LOX are depicted in Table 3.

Nitric oxide acts as a pro-inflammatory mediator. Pro-inflammatory stimuli can activate inducible nitric oxide synthase (iNOS). iNOS inhibition is an effective approach for anti-inflammatory treatments. iNOS activity inhibition also occurs in a dose-dependent manner. *G. conicarpa* shows moderate iNOS inhibiting activity.

In the cellular nitrite assay, the ability of *G. conicarpa* extract to decrease the level of nitrite in the cellular system was analysed. The level of nitrite was reduced from 715.77 ± 0.37 to 467.28 ± 0.58 µg/mL of cell lysate. LPS induced cells were treated with LC_{50} values (DFC: standard)

Table 3 LC_{50} value of COX and LOX

Activity	LC_{50}: STANDARD	LC_{50}: *G. conicarpa*
COX	15.7813 µg/mL	24.8217 µg/mL
LOX	4.4164 µg/mL	9.5330 µg/mL

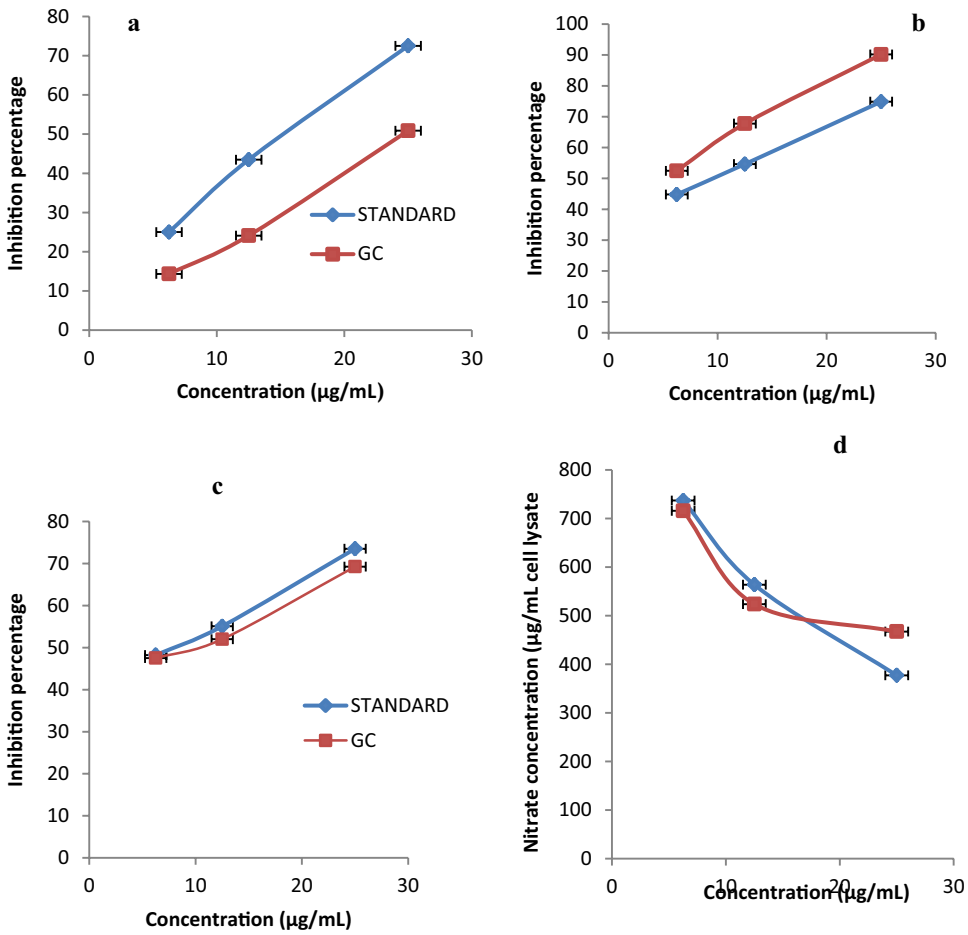


Fig. 5 **a** COX activity **b** LOX activity **c** iNOS activity **d** Cellular nitrate level

of 15.78137967 µg/mL and (*G. conicarpa*) 24.821711 µg/mL. The enzyme-linked immunosorbent test (ELISA) has been used to measure the expression of COX-2 and thereby the effectiveness of anti-inflammatory therapies. The extract reduced the COX-2 level in LPS-stimulated RAW cells from 0.84 ± 0.05 to 0.75 ± 0.05 U/mL, (Fig. 6). The decreased COX-2 enzyme level has a direct correlation with decreased iNOS activity and thereby cellular nitrate levels (Salem et al. 2022). Methanolic leaf extract of *Garcinia conicarpa* can be used as an effective anti-inflammatory agent. There are no available studies on the anti-inflammatory potential of *Garcinia conicarpa* yet.

Anticancerous activity

Certain compounds in medicinal plants can act as anti-cancer drugs by arresting cancer cell proliferation. Cytotoxicity was analysed in both normal L929 cells as well as MCF-7 cancer cell lines. In L929 cells, *G. conicarpa* exerts dose-dependent cytotoxicity, in which cell viability decreases with an increase in concentration (Fig. 7). Higher LC_{50} values (177.3720029 µg/mL) show lesser toxicity in normal cells (Fig. 8b). On the other hand, in cancer lines, even a lower LC_{50} (163.11334 µg/mL) value causes several apoptotic features like cell shrinkage, nuclear fragmentation, budding, etc. (Fig. 8d). Here, too, with an increase in concentration, cell viability reduces proportionately from 95.7% to 71.8%. So far, there seems to be no report regarding the anticancer activity of *Garcinia conicarpa* on the MCF-7 cell line.

Discussion

Surprisingly numerous compounds with antioxidant and anti-cancer properties are identified in plant materials. Drugs derived from phytocompounds are crucial for the battle against cancer, according to Vijayalakshmi et al. (2013). Polyisoprenylated benzophenones, polyphenols, bioflavonoids, xanthones, lactones, and triterpenes are the important phytocompounds isolated from *Garcinia* species. Methanolic leaf extract of *G. conicarpa* contain a wide variety of phytochemicals. β-caryophyllene, γ-cadinene, cyclosativene, α-copaene β-panasinsene, α-gurjunene, α-guaiene cis- muurola- 3,5- diene, *cis*- cadina-1(6),4- diene, amorpha- 4,11—diene, α-humulene, *cis*- cadina-1(6),4- diene, δ-selinene and *trans*- muurola- 4, (14)5–diene are some

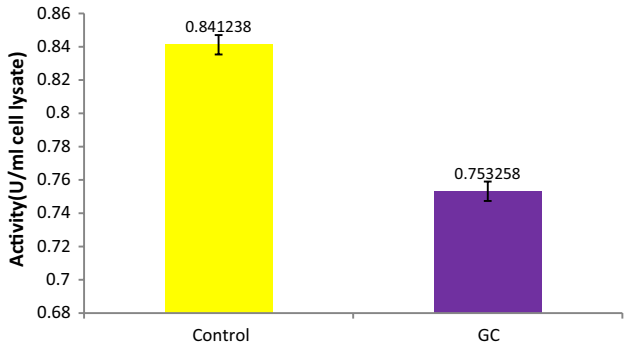


Fig. 6 Effect of *G. conicarpa* on COX-2 production

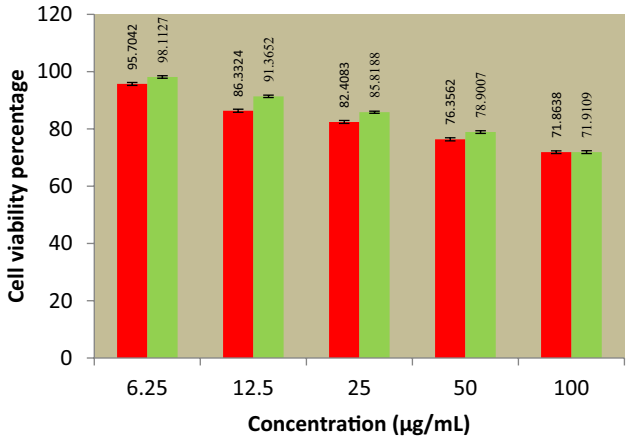


Fig. 7 Viability percentage of L929 and MCF-7 cell lines

of the leaf volatile chemicals obtained from *G. conicarpa* leaves in a previous study by Shameer et al. (2016). Literature review of some compounds identified through HR LC–MS such as zapotin, 7-dehydrologanin tetraacetate, nigakilactone B, and somniferin have anti-inflammatory activity. Compounds such as Zapotin, Somniferine and Nigakilactone B have antioxidant, anti-inflammtory and anticancerous activity.

Zapotin, a polymethoxyflavone isolated from *Punica granatum,* was used as an anti-inflammatory agent in traditional medicine. There were previous reports regarding the anticancer potential of Zapotin against human breast cancer (MCF-7), human glioma (U251N), human pancreatic cancer (PANC-1), and human colon cancer (H-116) cell lines (Strawa et al. 2021).

Flavanoids can scavenge different reactive oxygen species (ROS) and also inhibit COX and LOX enzymes involved in inflammation (Shen et al. 2022). Antioxidants can scavenge different free radicals and inhibit chains of oxidative reactions. Previous studies revealed higher antioxidant potential in different *Garcinia* species (Nguyen et al. 2021). Antioxidant activity was evaluated by DPPH, ABTS, hydroxyl, and nitric oxide free radical scavenging assays. In all the assays, *G. conicarpa* had higher to moderate activity, suggesting it is a good antioxidant agent. Flavonoids and phenolic acids are examples of polyphenolic compounds with health benefits. The phenolic acids class of substances includes 1-O-sinapoylglucose. Higher phenolic content in plants contributes to their ability to scavenge free radicals (Zhang

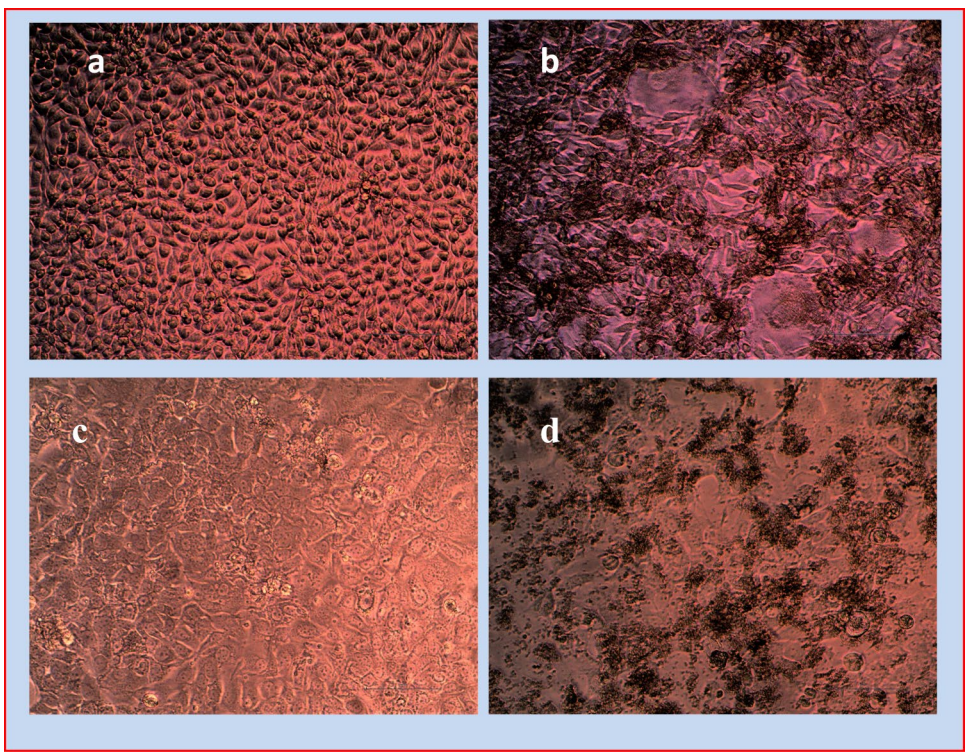


Fig. 8 Cytotoxic effect of *G. conicarpa* on L929 cells: (**a**) control (**b**) 100 µg/mL; MCF-7 Cell lines (**c**) control (**d**) 100 µg/mL

Table 4 *Garcinia* species and their activity

Sl.no	*Garcinia* species	Activities	References
1	*Garcinia mangostana*	Antioxidant, anticancer, anti-inflammatory	Abate et al. 2022
2	*Garcinia kola*	Antioxidant, anticancer, anti-inflammatory	Emmanuel et al. 2022
3	*Garcinia indica*	Antioxidant, anticancer, anti-inflammatory	Desai et al. 2022
4	*Garcinia atroviridis*	Antioxidant, Anticancer, Anti-inflammatory	Shahid et al. 2022

et al. 2020). Activation of AKT (protein kinase B), ERK (extracellular signal regulated kinase), p53, and other cellular pathways underlying apoptosis as well as the production of intracellular reactive oxygen species (ROS) can be inhibited by mangostanin, a Xanthone derived from the *Garcinia mangostana* fruit (Abate et al. 2022). In HR LC–MS, compounds with previous antioxidant activity reports such as 1-O-Sinapoylglucose, somniferine, Nigakilacton B, zapotin, and 7-dehydrologanin tetraacetate were also obtained. Plants with high antioxidant capacities should have better anti-inflammatory benefits. Table 4 shows previous reports of different biological activities shown by *Garcinia* species.

Inflammation is an immediate response to any injury. iNOS and COX-2 are two important inflammatory markers, and their inhibition helps in the prevention of inflammation. Garcinol, a polyisoprenylated benzophenone derivative from *Garcinia indica* fruit rind, has inhibitory action on iNOS and COX-2 by downregulating nuclear factor-kappa B (NF-B) induced by LPS (Liao et al. 2004). Nitric oxide-mediated inflammatory responses are alleviated by iNOS inhibitors; here, *G. conicarpa* showed a concentration dependent increase in the inhibition percentage of the enzyme. A reduction in iNOS activity also decreased NO production proportionately in the cells. COX-1, COX-2, and COX-3 are the 3 isoforms of the cyclooxygenase (COX) enzyme, among these, COX-2 is responsible for prostaglandin mediated inflammation by converting arachidonic acid to prostaglandins (Kalita et al. 2022).

Inhibitory actions on COX-2 and iNOS justify their application as anti-inflammatory agents. The antiinflammatory potential of *Garcinia* species on RAW 264.7 cell lines was validated in previous studies (Feng et al. 2021; Xue et al. 2020). Figure 9 depicts the iNOS activation and after effect of ROS.

Breast cancer is the most frequently diagnosed type of cancer among women. Current breast cancer therapeutic approaches have several limitations and side effects. In this scenario, natural products from plants with significant anti-breast cancer activities have to be studied. Through HR LC–MS, compounds like zapotin, nigakilactone B, pubesenolide, and euphorin against multiple cancer cells were obtained. There have been previous reports on the

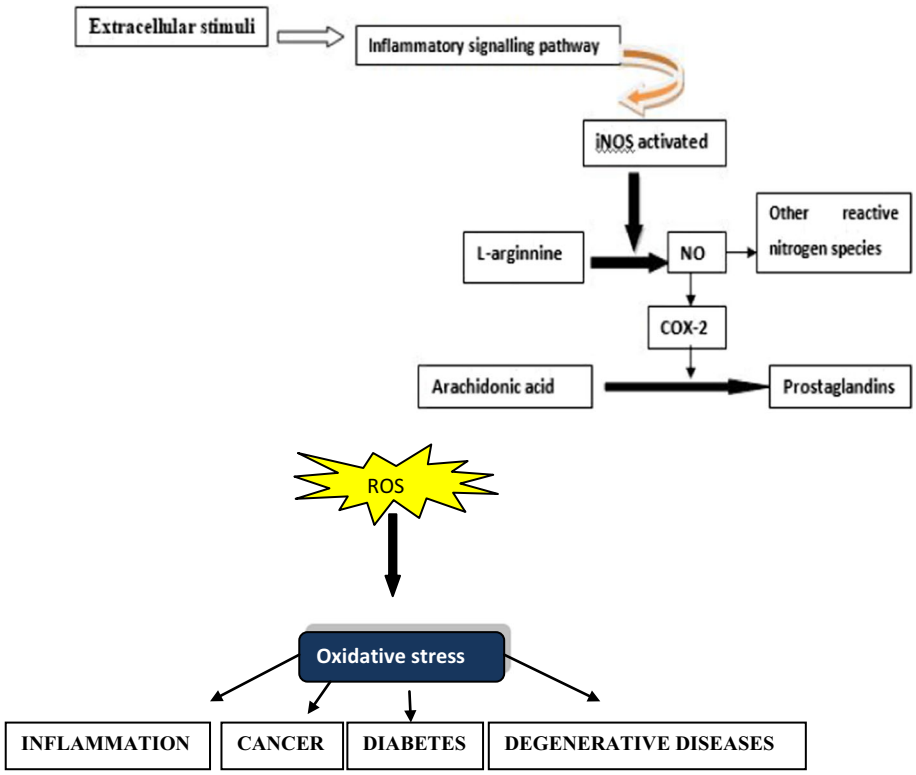


Fig. 9 Schematic illustration of iNOS activation and after effect of ROS

anticancer activities of various *Garcinia* species on MCF-7 cell lines (Brito et al. 2022). When the anticancer activity of *G. conicarpa* was evaluated in MCF-7 cell lines, cell viability decreased gradually with increasing concentration. Identification and screening of novel drugs from *G. conicarpa* with anti-breast cancer properties are required. In brief, all these results confirm that *Garcinia conicarpa* methanolic leaf extract has potent antioxidant effects that may contribute to its anti-inflammatory and anticancer properties, and conducting more studies on this topic is necessary. This is the first study to reveal the correlations between the phytochemical profiles, antioxidant and anti-inflammatory activities, as well as the anticancerous activities of *G.conicarpa*.

Conclusions

Phytochemical analysis, antioxidant, anti-inflammatory and anticancerous activities of *Garcinia conicarapa* were evaluated in the study for the first time. In this study, phytochemical analysis with HR LC–MS, the antioxidant assays with DPPH, ABTS, hydroxyl and nitric oxide free radical scavenging, ani-inflammatory activity with iNOS inhibition, LOX, COX activity, COX-2 expression and anticancer activity with MTT assay was conducted. *G. conicarpa* showed significant antioxidant and anti-inflammatory activities with very low toxic effects. Through further investigation, *G.conicarpa* can be used for the development of potential anti-inflammatory and chemotherapeutic drugs.

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Author contributions DMV—Idea for the article; DMV & JET—Literature search and data analysis; JET—Drafted and/or critically revised the work.

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Data availability Not applicable.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethics approval and consent to participate Not applicable.

Consent for publication All the authors have approved the manuscript for submission.

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