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In Vitro Study of Anti-Inflammatory and Anti-Oxidant Activity of Four Gingers with Various Solvent Extractions

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Authors
Revathy Leena Ravi, *MSc*[1*]
Janet Rani Rajiah, *PhD*[1]

[1] Department of Microbiology, Sadakathullah Appa College, Manonmaniam Sundaranar University, Rahmath Nagar, Tirunelveli-627011, Tirunelveli, Tamilnadu, India.

*** *Correspondence***
Department of Microbiology, Sadakathullah Appa College, Manonmaniam Sundaranar University, Rahmath Nagar, Tirunelveli-627011, Tirunelveli, Tamilnadu, India.
E-mail:revathyravi3363@sadakath.ac.in

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ABSTRACT

Background: Ginger research could lead to the discovery of its potential benefits for various applications, including medicinal therapy, nutrition, and pharmacology. This study aimed to analyze the anti-inflammatory and anti-oxidant activities of four ginger extracts using different polar and non-polar solvents. .

Materials & Methods: The dry powder (50 g) of four gingers was macerated using acetone, dimethyl sulfoxide, ethanol, methanol, chloroform, hexane, petroleum ether, and ethyl acetate and then placed on a rotating shaker (25 rpm) at 28 ℃ for 5 days until extraction. The prepared extracts underwent biological screening assays for anti-oxidant and anti-inflammatory properties.

Findings: The acetone extract of *Alpinia calcarata* had the lowest IC_{50} value for DPPH scavenging. The IC_{50} value of *A. calcarata* dimethyl sulfoxide extract was 89.59 µg/mL for ABTS activity. The IC_{50} value of *A. calcarata* acetone extract for hydrogen peroxide scavenging was 85.43 µg/mL. Evaluation of the anti-inflammatory potential of ginger extracts showed that the petroleum ether extract of *Zingiber zerumbet* had the lowest IC_{50} value of 90.95 g/mL for albumin stabilization compared to the other solvents.

Conclusion: The study compared solvent extracts of different gingers, revealing diverse bioactivity depending on the extraction method. *A. calcarata* and *Z. zerumbet* extracts showed strong antioxidant and anti-inflammatory properties. These extracts had lower IC_{50} values, demonstrating their efficacy in blocking proteinases, stabilizing cellular components, and scavenging free radicals. These results provide valuable insights into the pharmacological potential of these extracts as natural antioxidants and anti-inflammatory agents.

Keywords: Rhizomatous plants, *Zingiberaceae* family, Polar and non-polar extraction, Anti-oxidant and anti-inflammatory activities

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Introduction

Ginger (*Zingiber officinale Roscoe*) belongs to the *Zingiberaceae* family and the *Zingiber* genus. This genus has long been used both as a spice and as a medicine. Ginger root is widely used to reduce and treat many common ailments, including headaches, colds, nausea, and vomiting. Recent research has identified several bioactive compounds such as phenolic and terpene compounds in ginger, which are mostly gingerols, shogaols, and paradols. Ginger possesses biological activities such as antioxidant, anti-inflammatory, antimicrobial, and anticancer properties [1-3]. Antioxidant chemicals could regulate free radical generation and disrupt the oxidative stress pathway. Apart from their many medical applications, antioxidants help preserve food by prolonging its freshness. Furthermore, ginger has the ability to prevent and control many diseases like neurodegenerative and cardiovascular diseases, obesity, diabetes, chemotherapy-induced nausea, vomiting, respiratory disorders, and so on.

Research into the anti-inflammatory and antioxidant properties of ginger is significant because it joins traditional understanding with modern scientific research to enhance natural remedies for various health problems.

By focusing on the bioactive chemicals in ginger and their modes of action, researchers could gain useful insights to improve health outcomes, create new drugs, and improve public health practices. Ginger research helps identify ginger bioactive components with anti-inflammatory and antioxidant qualities as well as characterize their molecular and cellular mechanisms of action. Furthermore, it provides the possibility to assess ginger efficiency in reducing inflammation and oxidative stress in a variety of in vitro and in vivo models as well as to determine its ideal dosage and form to achieve the desired outcomes. A rhizomatous plant species known as *Alpinia calcarata* Roscoe categorized in the *Zingiberaceae* family is commonly used as a general therapeutic resource in Sri Lanka [1]. This plant is found in tropical countries like Malaysia, India, and Sri Lanka and has therapeutic effects on reducing swelling, soothing stomach ache, and stimulating the circulatory system [2, 3]. This plant is commonly used in the Indian subcontinent to treat respiratory problems, inflammatory disorders, coughs, and colds [4]. *A. calcarata* rhizomes are suggested as an aphrodisiac, and their decoction is frequently used to treat conditions like bronchitis, diabetes, respiratory conditions, etc. [5]

Curcuma amada Roxb., also known as mango ginger, is an aromatic perennial herb of the *Zingiberaceae* family, which consists of 70 to 80 rhizomatous species [8, 9], originating in the Indo-Malayan region but also found in some other countries [10]. Mango ginger has a raw mango flavor, which is used in pickles and other food preparations. *C. amada* is a distinctive spice that resembles ginger (*Z. officinale*) in appearance but tastes like fresh mango (*Mangifera indica*). Linnaeus coined the genus *Curcuma* in his book entitled "Species *Plantarum*" in 1753. The Arabic word "kurkum", which means yellow, is most likely the source of this name [6, 7]. Because of its many therapeutic benefits, mango ginger is highly valued in Ayurveda and Unani medical systems. Additionally, it has biological activities such as CNS (central nervous system) depressant, antibacterial, antifungal, antioxidant (AO), and anti-inflammatory (AI) effects. Its main chemical constituents are starch, terpenoids, volatile oils, phenolic acids, and curcuminoids [11].

Kaempferia galanga is the dried rhizome of *Kaempferia galanga* L. [12, 13], which is mainly found in India, China, Myanmar, Indonesia, Malaysia, and Thailand. It contains bioactive

compounds that are utilized in conventional medicine to diagnose and cure tumors, rheumatism, colic, inflammation, and dry cough [14-17] and also in some countries to treat urticarial infections and intestinal wounds [18, 19]. Various compounds found in phytochemical studies with AI, AO, anti-tumor, and anti-angiogenic properties include terpenoids, phenolics, diarylheptanoids, and flavonoids [20-24].

It is well-known that Asia and the Pacific Islands are the main habitats of medicinal plants of the genus *Zingiber*, which belongs to the *Zingiberaceae* family [25, 26]. The rhizome of *Z. zerumbet* is commonly utilized as a herbal remedy for fever, pain, and other conditions in many countries [27, 28]. Some studies have revealed that the combination of this rhizome powder and *Morinda citrifolia* is useful for pain relief [29-31]. In addition, zerumbone, a natural sesquiterpene extracted from *Z. zerumbet,* is an effective compound with potential AO and anticancer properties, prompting further investigation on its chondroprotective properties against osteoarthritis [32]. Furthermore, the medicinal herb *Z. zerumbet* has been found to have AI, anti-cancer, and AO characteristics [33-35].

Objectives: The purpose of this study was to examine the anti-inflammatory and anti-oxidant activities of different solvent extracts of four gingers, leading to effective therapeutic applications in medicine and pharmaceuticals.

Material and Methods

Collection and preparation of sample extracts: For maceration extraction, approximately 50 g of dry powder of *A. calcarata* (*Ac*), *C. amada* (*Ca*), *K. galanga* L. (*Kg*), and *Z. zerumbet* (*Zz*) were mixed with 50 mL of acetone, dimethyl sulfoxide (DMSO), ethanol, methanol, chloroform, hexane, petroleum ether, and ethyl acetate. The ginger samples were extracted employing polar and non-polar solvents on a revolving shaker at 28 °C for 5 days and then subjected to biological screening assays for AI and AO activities.

In this study, serial concentrations (20, 40, 60, 80, and 100 µg/mL) were used in different assays. Many studies have used higher concentrations to find the lowest concentration needed to produce a biological effect. These steps are essential for establishing dose-response relationships, finding effective doses, understanding how drugs work, evaluating safety, improving experimental design, and making rational comparisons. This systematic approach ensures that the research findings are reliable, interpretable, and usable for practical applications in various fields of study.

Experimental replicates: Each concentration group underwent experimental replications. Repeated experiments are more likely to accurately quantify variables, ensure statistical validity, detect errors or outliers, assess consistency and reproducibility, improve generalizability of results, and strictly adhere to peer review and scientific research guidelines.

Data analysis plan: Experimental results were presented as mean ± SEM (standard error of the mean) of three parallel measurements. Analysis of variance (ANOVA) test and then Newman-Keuls multiple comparison test were performed at a 5% significance level using Agricolae package in R software to evaluate IC_{50} (half-maximal inhibitory concentration) values.

Evaluation of AO potential DPPH (2,2-diphenyl-1-picrylhydrazyl) assay: DPPH assay is a widely used method to evaluate the antioxidant properties of substances by measuring their ability to scavenge DPPH radicals, providing valuable information in fields such as food science, pharmacology, and cosmetics. The free

radical scavenging activity of the prepared extracts was assessed in vitro by DPPH assay based on the standard method [36, 37]. The stock solution was set by dissolving 24 mg of DPPH in 100 mL of methanol and stored at 4 to 20 °C. A solution of 0.2 mM DPPH in methanol was prepared, then 4 mL of *Ac, Ca, Kg,* and *Zz* extracts at various concentrations (20, 40, 60, 80, and 100 µg/mL) were mixed with 1 mL of the prepared methanol solution comprising DPPH radicals. After vigorously shaking the mixture and allowing it to stand for half an hour, the absorbance at 517 nm was determined. Ascorbic acid was applied as a positive control, and DPPH solution without sample was employed as a negative control (blank). The scavenging activity was calculated based on the proportion of DPPH radicals scavenged using the following equation:

(1)

$$\text{Percentage of inhibition} = [(\text{control OD} - \text{sample OD}) / (\text{control OD})] \times 100$$

Hydrogen peroxide-scavenging activity: The hydrogen peroxide-scavenging activity assay measures the ability of substances to neutralize hydrogen peroxide, thereby preventing oxidative damage and reflecting their antioxidant potential.

Nabavi et al. (2009) [38] explained H_2O_2 scavenging activity by generating a mixture with hydrogen peroxide solution (2 mmol/L) in phosphate buffer (pH 7.4). In the current study, *Ac, Ca, Kg,* and *Zz* extracts (1 mL) at different concentrations (20, 40, 60, 80, and 100 µg/mL) were added to hydrogen peroxide solution (0.6 mL). Ascorbic acid was applied as a positive control, and H_2O_2 without sample was employed as a negative control (blank). The absorbance of hydrogen peroxide at 230 nm was measured after 10 min using a UV visible spectrometer (Shimadzu UV-1800, Japan). By employing Eq. (1), the percentage of H_2O_2 scavenging activity was calculated.

ABTS(2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging assay: ABTS radical scavenging assay assesses the ability of substances to neutralize ABTS·+ radicals, reflecting their antioxidant potential by measuring the decrease in absorbance at 734 nm. ABTS radical cation decolorization assay is utilized to find the free radical scavenging activity of plant samples [39]. ABTS·+ radicals were prepared by mixing 7 mM ABTS and 2.45 mM potassium persulfate solutions in equal quantities at room temperature for 12-16 hours, the resulting solution (10 mL) was then diluted with methanol (20 mL) to obtain an absorbance of 0.700 at 734 nm. Ascorbic acid was applied as a positive control, and ABTS without sample was employed as a negative control (blank). After adding *Ac, Ca, Kg,* and *Zz* extracts (1 mL) at different concentrations (20, 40, 60, 80, and 100 µg/mL) to 3.995 mL of diluted $ABTS^{\cdot+}$ solution, the absorbance was recorded 30 min after the initial mixing. Each assay was conducted in parallel with the desired solvent as a blank. At least three repeated experiments were performed, and the percentage (%) of radical scavenging activity was calculated by measuring the decrease in absorbance at 734 nm using the following formula (2):

(2)

$$ABTS^{\cdot+} \text{ scavenging effect } (\%) = ((Abs_B - Abs_A)/Abs_B) \times 100$$

Where Abs_B is absorbance of ABTS radical + methanol, and Abs_A is absorbance of ABTS radical + sample extract/standard. Trolox was utilized as a standard substance.

Screening of anti-inflammation (AI) property Albumin denaturation inhibition assay: Denaturation assays are frequently employed to evaluate the unfolding and stability properties of proteins, including albumin. These assays typically involve exposing the protein to various stress conditions and then measuring changes in its structural integrity

or functional properties. In the study, the AI properties of *Ac, Ca, Kg,* and *Zz* extracts at various concentrations (20, 40, 60, 80, and 100 µg/mL) were investigated using the albumin denaturation inhibition technique described by Mizushima and Kobayashi (1968) as well as Sakat and colleagues (2010) [40, 41]. The reaction mixture was composed of test extracts (1 mL) and bovine albumin fraction (0.5 mL of 1% aqueous solution), adjusted with 1N HCl. Diclofenac sodium was applied as a positive control, and reaction mixture without sample was used as a negative control (blank). The reaction mixture was incubated at 37 °C for 20 min and then heated to 51 °C for 20 min. After cooling, the turbidity was measured at 660 nm using a UV-visible spectrophotometer (Model 119, Systronic). The experiment was carried out in triplicate, and the inhibition percentage of protein denaturation was evaluated as follows:

(3)

$$\text{Percentage of inhibition} = (Abs_{control} - Abs_{sample}) \times 100 / Abs_{control}$$

Membrane stabilization Preparation of red blood cell (RBCs) suspension : The blood sample of a healthy sheep that had not received nonsteroidal anti-inflammatory drugs (NSAIDs) since two weeks prior to sampling was taken and transferred to centrifuge tubes. The tubes underwent centrifugation at 3000 rpm for 10 min, followed by three rinses with normal saline, and the blood volume was determined and reorganized as a 10% v/v suspension.

Heat induced hemolysis: Heat-induced hemolysis assays are useful tests for researching how temperature stress affects cell membrane integrity, especially in red blood cells.

Their quantitative measurements are useful in evaluating the protective effects of different compounds or conditions on membrane stability. The reaction mixture (2 mL) included *Ac, Ca, Kg,* and *Zz* extracts at various concentrations (20, 40, 60, 80, and 100 µg/mL) and 1 mL of 10% erythrocyte suspension. Instead of the test sample, only saline solution was added to the control test tube.

The reaction mixture of haem suspension at neutral pH and RT (room temperature) without sample was used as a negative control (blank).

The reaction mixture in each centrifuge tube was incubated at 56 °C for 30 min in a water bath. Following incubation, the reaction mixture was centrifuged at 2500 rpm for 5 min, the tubes were subcooled, and the absorbance of the supernatant was measured at 560 nm. For each test sample, the experiment was conducted three times [41-43].

Proteinase inhibitory activity: The basis of proteinase inhibitory activity assays is to measure the capacity of inhibitors to obstruct the catalytic activity of proteases. This information is essential for comprehending the mechanisms underlying enzyme inhibition and locating possible therapeutic agents.

Different extracts of *Ac, Ca, Kg,* and *Zz* at various concentrations (20, 40, 60, 80, and 100 µg/mL) were utilized in this experiment following the method described by Leelaprakash and Dass (2011) [37]. The reaction mixture (2 mL) comprised 0.06 mg of trypsin, 1 mL of 20 Mm Tris HCl buffer (pH 7.4), and 1 mL of test sample in different concentrations. Diclofenac sodium was applied as a positive control, and the reaction mixture without sample was used as a negative control (blank).

The mixture was incubated at 37 ºC for 5 min. Then 1 mL of 0.8% (w/v) casein was added. The mixture was incubated for another 20 min. After stopping the reaction with 2 mL of 70% perchloric acid, the cloudy suspension was centrifuged at 3000 rpm for 10 min, and the absorbance was measured. The absorbance was recorded using a UV-visible

spectrophotometer (Model 119, Systronics) against buffer as blank.

Findings

Evaluation of AO potential DPPH assay: DPPH assay was used to evaluate the scavenging activity of different ginger extracts (*Ac, Ca, Kg,* and *Zz*) in polar and non-polar solvents, such as acetone, DMSO, ethanol, methanol, chloroform, hexane, petroleum ether, and ethyl acetate.

Each solvent system has a unique solubility property for extracting phytochemicals from plant parts. Among *Ac* extracts, its acetone extract (100 µg/mL) with the lowest IC_{50} value of 83.31 µg/mL showed the maximum DPPH-scavenging activity of 59.52 ± 0.55% compared to the other solvents (p= .329922). Furthermore, the DMSO extract of *Ca* (100 µg/mL) exhibited an inhibitory activity of 36.5 ± 0.25% with the lowest IC_{50} value of 144.85 µg/mL (p= .40606). An inhibitory activity of 32.5% (100 µg/mL) was obtained for *Kg* in both polar (methanol and DMSO) and non-polar (chloroform and hexane) solvents; among the eight solvents of *Kg*, its ethyl acetate extract showed the lowest IC_{50} value of 148.27 µg/mL (p= .48099). In addition, the acetone extract of *Zz* (100 µg/mL) showed an inhibitory activity of 56.5 ± 0.55% with the

Table 1) Half-maximal inhibitory concentrations (IC_{50}) of four ginger extracts in various solvents in DPPH assay

Solvents	*Ac*	*Ca*	*Kg*	*Zz*
Acetone	83.31 [a]	228.77 [c]	248.54 [c]	87.47 [a]
Ethanol	164.04 [b]	162.82 [b]	163.77 [b]	183.79 [b]
Methanol	183.32 [b]	184.44 [b]	184.19 [b]	198.1 [b]
DMSO	99.74 [a]	144.85 [b]	186.89 [b]	95.6 [a]
Chloroform	120.32 [b]	191.55 [b]	181.51 [b]	94.28 [a]
Hexane	94.31 [a]	175.05 [b]	177.53 [b]	96.72 [a]
Ethyl acetate	151.08 [b]	246.52 [c]	148.27 [b]	128.51 [b]
Petroleum ether	203.59 [c]	200.4 [c]	203.59 [c]	213.55 [c]

a: good IC50 value < 100, b: moderate IC50 value < 200, and c: poor IC200< 50

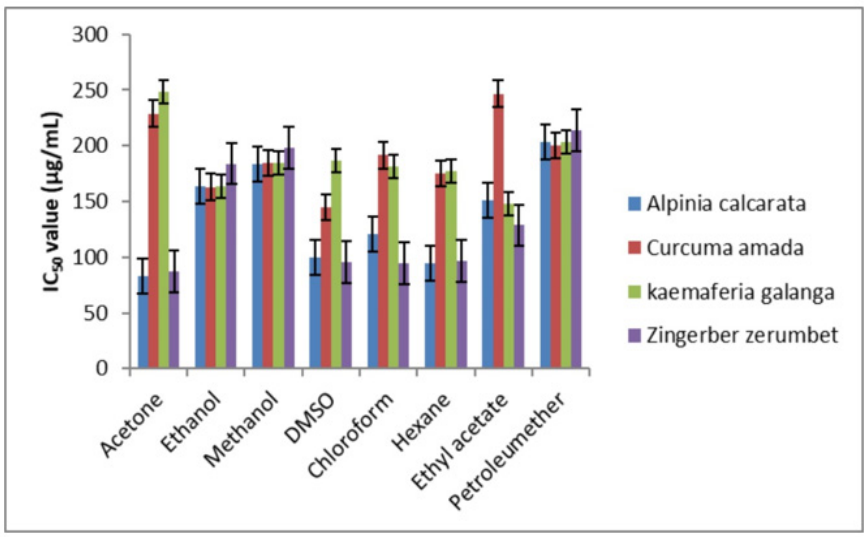

Figure 1) Schematic presentation of half-maximal inhibitory concentrations (IC_{50}) of four ginger extracts in various solvents in DPPH assay

lowest IC_{50} value of 87.47 µg/mL (p= .274534). IC_{50} values of different solvent systems are described in Table 1 and Figure 1.

Hydrogen peroxide-scavenging activity: Hydrogen peroxide is a free radical, which damages cells and leads to cellular oxidative stress. This assay was used to assess the inhibitory activity of different ginger extracts (*Ac, Ca, Kg,* and *Zz*) in polar and non-polar solvents, such as acetone, DMSO, ethanol, methanol, chloroform, hexane, petroleum ether, and ethyl acetate. Among *Ac* extracts, its DMSO extract (100 µg/mL) with an IC_{50} value of 86.26 µg/mL showed the maximum inhibitory activity of 57.2 ± 0.12% compared to the other solvents (p= .661822, ANOVA). Furthermore, *Ca* petroleum ether extract (100 µg/mL) exhibited an inhibitory activity of 37.5 ± 0.10% with the lowest IC_{50} value of 136.31 µg/mL (p= .691652). An inhibitory activity of 38.5 ± 0.14% was obtained for *Kg* petroleum ether extract (100 µg/mL); among the eight solvents of *Kg*, it petroleum ether extract showed the lowest IC_{50} value of 126.55 µg/mL (p= .714644). In addition, the DMSO extract of *Zz* (100 µg/mL) showed an inhibitory activity of 57.2 ± 0.12% with an IC_{50} value of 94.71 µg/mL (p= .640337). IC_{50}

Table 2) Half-maximal inhibitory concentrations (IC_{50}) of four ginger extracts in various solvents in hydrogen peroxide-scavenging assay

Solvents	*Ac*	*Ca*	*Kg*	*Zz*
Acetone	85.43 [a]	188.45 [b]	176.38 [b]	94.22 [a]
Ethanol	146.46 [b]	150.89 [b]	154.85 [b]	154.58 [b]
Methanol	144.44 [b]	171.99 [b]	169.03 [b]	201.02 [c]
DMSO	86.26 [a]	179.55 [b]	164.77 [b]	94.71 [a]
Chloroform	124.74 [b]	152.07 [b]	139.82 [b]	108.45 [b]
Hexane	91.68	185.26 [b]	189.27 [b]	98.32 [a]
Ethyl acetate	143.94 [b]	196.79 [b]	193.78 [b]	156.61 [b]
Petroleum ether	143.14 [b]	136.31 [b]	126.55 [b]	128.62 [b]

a: good IC50 value < 100, b: moderate IC50 value < 200, and c: poor IC200< 50

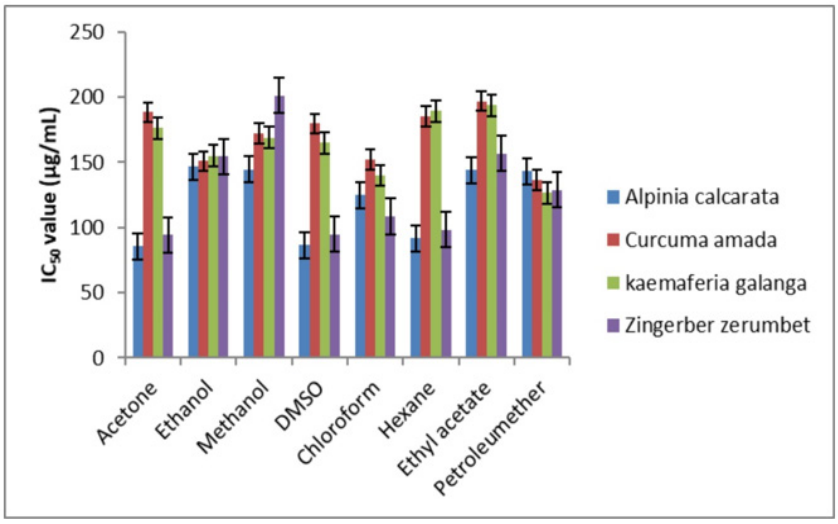

Figure 2) Schematic presentation of half-maximal inhibitory concentrations (IC_{50}) of four ginger extracts in various solvents in hydrogen peroxide-scavenging assay

values of different solvent systems are described in Table 2 and Figure 2.

ABTS radical scavenging assay: Strong oxidizing agents generated ABTS+ radicals, and the potential AO effects of hydrogen-donating compounds in the tested ginger extracts were measured to scavenge them. Among *Ac* extracts, its DMSO extract (100 µg/mL) with the lowest IC_{50} value of 89.59 µg/mL showed the maximum inhibitory activity of 55.1 ± 1.2% compared to the other solvents (p= .738417). *Ca* extracts (100 µg/mL) in methanol and chloroform solvents showed an inhibitory activity of 35.1 ± 1.12% with IC_{50} values of 173.24 (methanol) and 146.89 µg/mL (chloroform), respectively (p= .708223). *Kg* chloroform extract had the lowest IC_{50} value (141.49 µg/mL) and the highest inhibitory activity (36.1 ± 1.27%) among the eight *Kg* solvents (p= .716099). Furthermore, the DMSO extract of *Zz* had the lowest IC_{50} value of 92.39 µg/mL (p= .723905) compared to the other solvent systems. IC_{50} values of different solvent systems are shown in Table 3 and Figure 3.

AI effects of different gingers Albumin denaturation inhibition assay: The inhibitory activity against albumin denaturation represents the anti-inflammatory property of active plant principles. The petroleum

Table 3) Half-maximal inhibitory concentrations (IC_{50}) of four ginger extracts in various solvents in ABTS assay

Solvents	*Ac*	*Ca*	*Kg*	*Zz*
Acetone	94.29 [a]	222.93 [c]	217.57 [c]	100.6 [b]
Ethanol	171.71 [b]	168.13 [b]	168.76 [b]	192.62 [b]
Methanol	212.12 [c]	173.24 [b]	212.12 [c]	192.62 [b]
DMSO	89.59	207.27 [c]	205 [c]	92.39 [a]
Chloroform	110.17 [b]	146.89 [b]	141.49 [b]	110.42 [b]
Hexane	94.5 [a]	186.97 [b]	220.9 [c]	96.67 [a]
Ethyl acetate	127.8 [b]	211.73 [c]	196.03 [b]	127.77 [b]
Petroleum ether	150.81 [b]	169.51 [b]	150.81 [b]	247.55 [c]

a: good IC50 value < 100, b: moderate IC50 value < 200, and c: poor IC200< 50

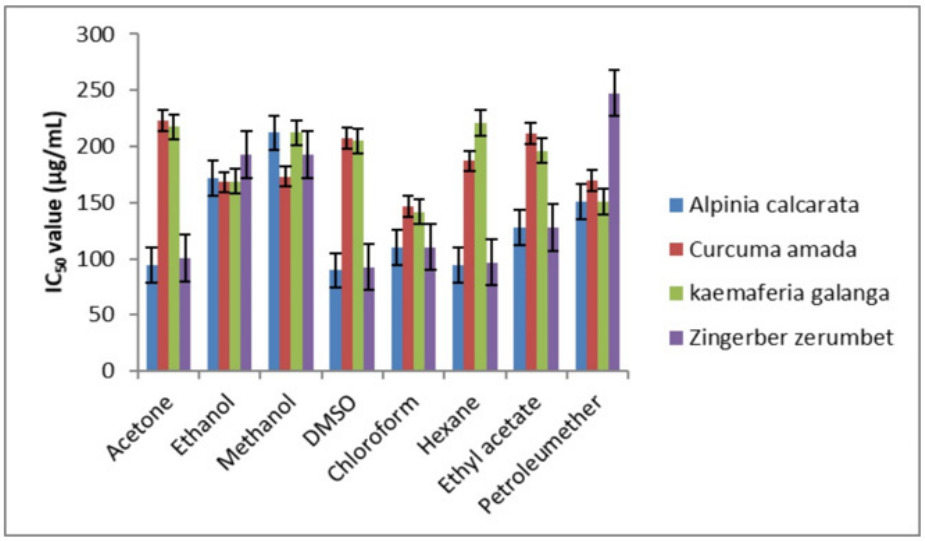

Figure 3) Schematic presentation of half-maximal inhibitory concentrations (IC_{50}) of four ginger extracts in various solvents in ABTS assay

ether extract of *Ac* (100 µg/mL) showed the maximum inhibitory activity of 53.65 ± 0.08% with the lowest IC_{50} value of 91.08 µg/mL compared to the other solvents (p= .435454). Furthermore, the DMSO extract of *Ca* (100 µg/mL) exhibited an inhibitory activity of 28.7 ± 0.04% with an IC_{50} value of 175.86 µg/mL (p= .87311). The maximum inhibitory activity of 29.7 ± 0.05% was obtained for *Kg* ethanol extract (100 µg/mL); among the eight solvents of *Kg,* its ethanol extract showed the lowest IC_{50} value of 169.75 µg/mL (p= .925347). In addition, the petroleum ether extract of *Zz* (100 µg/mL) showed a potential inhibitory activity of 56.65 ± 0.08% with the lowest IC_{50} value of 90.95 µg/mL (p= .574116). IC_{50} values of different solvent systems are described in Table 4 and Figure 4.

Heat-induced membrane stabilization assay: The AI effect is evaluated by heat-induced hemolysis with the principle of red blood cell membrane stabilization (HRBS assay). This facilitates the understanding of the local inflammatory response to the test extracts. A series of four different ginger plant extracts were evaluated to analyze their effect on RBC membrane stabilization

Table 4) Half-maximal inhibitory concentrations (IC_{50}) of four ginger extracts in various solvents in albumin denaturation assay

Solvents	*Ac*	*Ca*	*Kg*	*Zz*
Acetone	111.5 [b]	183.58 [b]	179.72 [b]	96.99 [a]
Ethanol	129.36 [b]	171.73 [b]	169.75 [b]	147.55 [b]
Methanol	113.31 [b]	216.87 [c]	207.77 [c]	119.18 [b]
DMSO	100.14 [b]	175.86 [b]	171.24 [b]	107.33 [b]
Chloroform	155.9 [b]	206.05 [c]	209.23 [c]	145.08 [b]
Hexane	168.29 [b]	159.05 [b]	233.31 [c]	180.63 [b]
Ethyl acetate	94.21 [a]	216.19 [c]	213.6 [c]	95.79 [a]
Petroleum ether	91.08 [a]	257.65 [c]	216.31 [c]	90.95 [a]

a: good IC50 value < 100, b: moderate IC50 value < 200, and c: poor IC200< 50

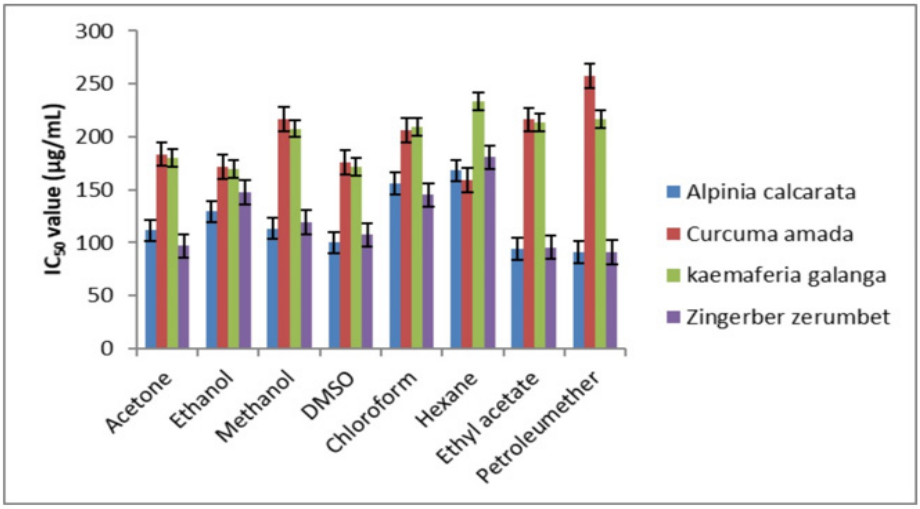

Figure 4) Schematic presentation of half-maximal inhibitory concentrations (IC_{50}) of four ginger extracts in various solvents in albumin denaturation assay

and lysis inhibition. The petroleum ether extract of *Ac* (100 µg/mL) showed the maximum inhibitory activity of 53.9 ± 0.23% with the lowest IC_{50} value of 85.83 µg/mL compared to the other solvents (p= .179908). Moreover, the acetone extract of *Ca* (100 µg/mL) exhibited an inhibitory activity of 28.5 ± 0.18% with an IC_{50} value of 181.72 µg/mL (p= .881317). The maximum inhibitory activity of 29.6 ± 0.18% was obtained for *Kg* ethanol extract (100 µg/mL); among the eight solvents of *Kg*, its ethanol extract showed the lowest IC_{50} value of 174.94 µg/mL (p= .879398). Additionally, the petroleum ether extract of *Zz* (100 µg/mL) showed a potential inhibitory activity of 59.9 ± 0.23% with the lowest IC_{50} value of 83.53 µg/mL (p= .261637). IC_{50} values of different solvent systems are explained in Table 5 and Figure 5.

Proteinase inhibition assay: Another in vitro screening for evaluating the AI properties of four ginger extracts in polar and non-polar solvents was to investigate their effect on inhibiting proteinase. Among *Ac* extracts, its petroleum ether extract (100

Table 5) Half-maximal inhibitory concentrations (IC_{50}) of four ginger extracts in various solvents in HRBS assay

Solvents	*Ac*	*Ca*	*Kg*	*Zz*
Acetone	103.02 [b]	181.72 [b]	177.7 [b]	89.57 [a]
Ethanol	147.9 [b]	179.18 [b]	174.94 [b]	155.1 [b]
Methanol	132.14 [b]	192.21 [b]	204.03 [c]	106.05 [b]
DMSO	86.28 [a]	203.85 [c]	201.97 [c]	93 [a]
Chloroform	142.92 [b]	185.56 [b]	196.97 [b]	176.21 [b]
Hexane	151.14 [b]	257.9 [c]	202.32 [c]	161.56 [b]
Ethyl acetate	91.94 [a]	219.47 [c]	220.4 [c]	96.06 [a]
Petroleum ether	85.83 [a]	214.71 [c]	219.16 [c]	83.53 [a]

a: good IC50 value < 100, b: moderate IC50 value < 200, and c: poor IC200< 50

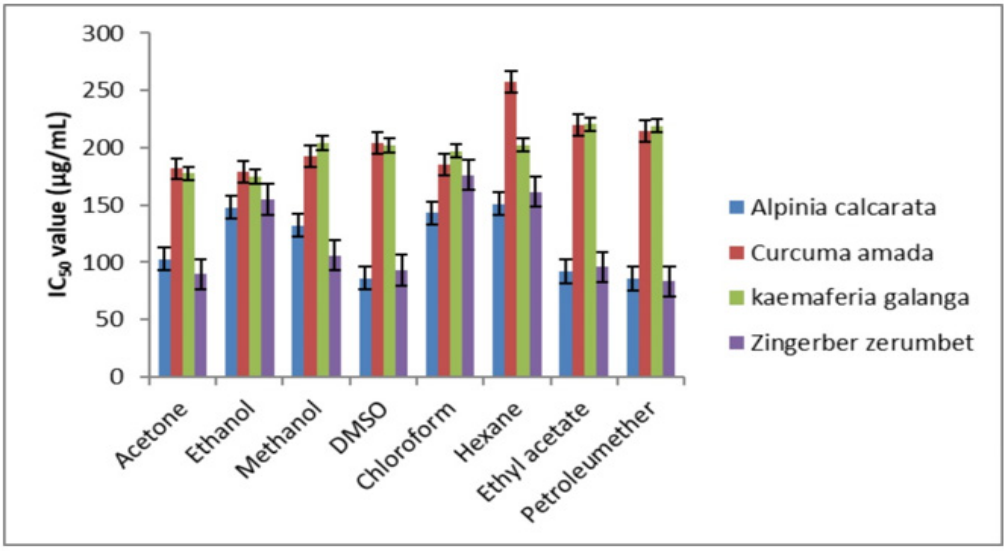

Figure 5) Schematic presentation of half-maximal inhibitory concentrations (IC_{50}) of four ginger extracts in various solvents in HRBS assay

µg/mL) showed the maximum inhibitory activity of 58.2 ± 0.28% with the lowest IC_{50} value of 82.56 µg/mL compared to the other solvents (p= .372272). Whereas DMSO, chloroform, and hexane extracts of *Ca* (100 µg/mL) exhibited a similar inhibitory activity of 28.9 ± 0.12%, with the lowest IC_{50} value of 170.82 µg/mL obtained from its DMSO extract (p= .89923). The maximum inhibitory activity of 29.98 ± 0.14% was obtained for *Kg* (100 µg/mL) in hexane; among the eight solvents of *Kg,* its DMSO extract showed the lowest IC_{50} value of 160.55 µg/mL (p= .900442). In addition, the petroleum ether extract of *Zz* (100 µg/mL) showed a potential inhibitory activity of 55.2 ± 0.28% with the lowest IC_{50} value of 88.59 µg/mL (p= .793747). IC_{50} values of different solvent systems are described in Table 6 and Figure 6.

Discussion

In a study by Arambewela and colleagues (2010) [44], the extracted oil from *Ac* rhizome exhibited an IC_{50} value of 45 ± 0.4 µg/mL for DPPH radical scavenging and provided a promising source of AO principles. Abirami

Table 6) Half-maximal inhibitory concentrations (IC_{50}) of four ginger extracts in various solvents in proteinase inhibition assay

Solvents	*Ac*	*Ca*	*Kg*	*Zz*
Acetone	111.62 [b]	194.24 [b]	190.37 [b]	95.65 [a]
Ethanol	147.02 [b]	224.75 [c]	225.66 [c]	141.21 [b]
Methanol	116.19 [b]	214.45 [c]	271.17 [c]	94.75 [a]
DMSO	96.37 [a]	170.82 [b]	160.55 [b]	104.26 [b]
Chloroform	157.53 [b]	191.77 [b]	198.5 [b]	142.39 [b]
Hexane	147.87 [b]	193.17 [b]	173.52 [b]	158.21 [b]
Ethyl acetate	99.39 [a]	233.85 [c]	261.2 [c]	113.18 [b]
Petroleum ether	82.56 [a]	208.43 [c]	203.82 [c]	88.59 [a]

a: good IC50 value < 100, b: moderate IC50 value < 200, and c: poor IC200< 50

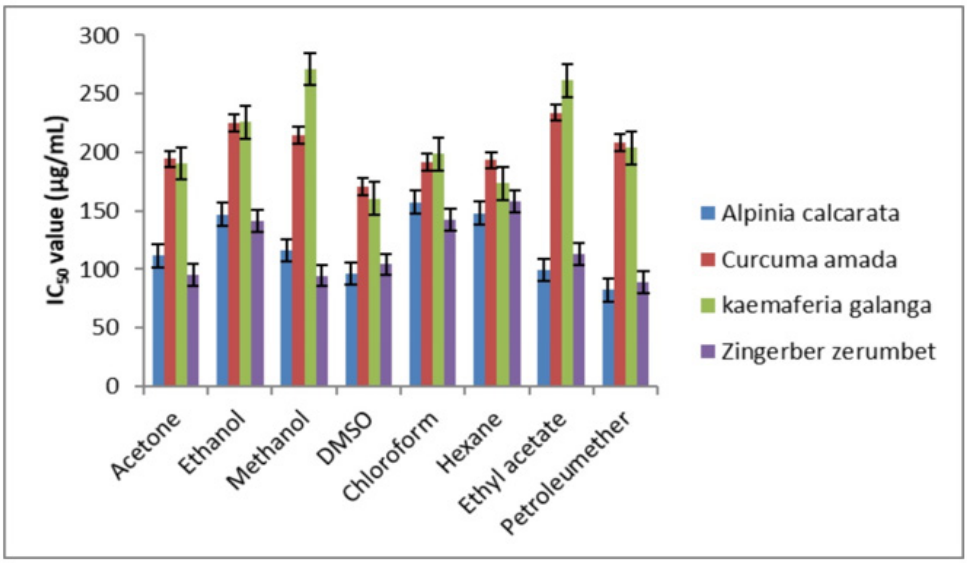

Figure 6) Schematic presentation of half-maximal inhibitory concentrations (IC_{50}) of four ginger extracts in various solvents in proteinase inhibition assay

et al. (2019) [45] reported that the methanol extract of *Curcuma* was more active for DPPH radical scavenging (83.9% at 200 µg/mL), although its aqueous extract also showed (58.01% at 200 µg/mL) scavenging activity. The petroleum ether extract of *Ca* in another study showed a better and lower IC_{50} value (18.98 ± 0.05 µg/mL) for scavenging DPPH radicals [46]. The best DPPH scavenging activity was observed in a study with IC_{50} values of 26-30 and 25-29 µg/mL of petroleum ether and ethanol extracts of *Ca* compared to other solvents; also, *Kg* essential oil was assessed to eliminate DPPH free radicals and showed better IC_{50} values (19.77 ± 1.28 µg/mL) [47]. Assiry et al. (2023) reported that the aqueous extract of *Zz* (1 mg/mL) showed the maximum scavenging activity of 90.6 ± 0.20%, whereas its ethanol extract (1 mg/mL) revealed a scavenging activity of 54.5 ± 0.76% [48].

The ethanol extract of *Ac* rhizomes exhibited the best hydrogen peroxide scavenging activity compared to the standard (ascorbic acid) in a research documented by Akhil (2017) [49]. In a study by Wang et al. (2023) [47], the maximum hydrogen peroxide scavenging activity was obtained using the methanol extract of *Ca* rhizomes with an IC_{50} value of 35 ± 0.62 µg/mL; also, hydroxyl radicals were scavenged by *Kg* essential oil (KGEO) with an IC_{50} value of 3.09 ± 0.34 mg/mL.

The ethanol extract of *Ac* exhibited a lower IC_{50} value of 141.75 µg/mL than its methanol (146.25 µg/mL) and aqueous (237.1 µg/mL) extracts in a study by Abirami et al. (2019) [45]. Ali et al. (2022) [50] showed that *Ca* rhizome ethanol extract at different concentrations (25, 50, 100, 200, 300, 400, and 500 µg/m) showed inhibitory activities (13.2, 25.6, 29.2, 50.8, 76.8, 89.2, and 99.6%, respectively). In their study, *Kg* essential oil exhibited potent scavenging activity on ABTS radicals in vitro with an IC_{50} value of 1.41 ± 0.01 µg/mL, and the methanol extract of *Zz* rhizome with potent AO compounds showed the lowest IC_{50} value of 11.38 ± 1.39 µg/mL.

In a study by Borah et al. (2019) [51], the ethanol extract of *Ac* exhibited potent inhibitory activity against albumin denaturation (93.46% at 500 µg/mL) with an IC_{50} value of 280.10 µg/mL, and the hydrodistillation extract of *C. caesia* Roxb leaves exhibited AI activity with an IC_{50} value of 182.5 µg/mL. In an in-vitro study by Ullah et al. (2014) [52], the ethanol extract of *C. zedoaria* at 500, 400, 300, 200, and 100 µg/mL doses significantly inhibited protein denaturation by 77.15, 64.43, 53.04, 36.78, and 23.70% , respectively.

Additionally, Begum et al. (2023) [53] showed that the essential oil derived from *Kg* rhizomes (KMCKG) rich in ethyl p-methoxycinnamate exhibited similar efficacy in inhibiting albumin denaturation with an IC_{50} value of 2.93 ± 0.59 µg/mL. Similarly, the ethanol extract of *Zz* has been shown to be effective in inhibiting albumin denaturation compared to diclofenac sodium (a positive control) [54]. A recent study reported that the ethanol extract of *Ac* (500 µg/mL) exhibited a potent heat-induced RBC membrane stabilization activity of 75.59% with an IC_{50} value of 355.71 µg/mL [5]. Arambewela et al. (2004) [5] found that the ethanol extract of *Ac* rhizomes (500 mg/kg) exhibited a better AI response in a carrageenan-induced inflammatory model four hours after carrageenan administration ($p< 0.05$) due to *Ac*-mediated effect on histamine and prostaglandin synthesis.

Zhou et al. (2015) [22] found that the lipopolysaccharide (LPS)-stimulated inflammatory response mediated by HMC-1 mast cells was significantly inhibited by kaempferol, shown by the MTT experiment. In their study, significant reductions were observed in the production of IL-6, IL-8, IL-1β, and TNF-α at a dosage of 40 µmol/L.

Different *Kg* extracts have been reported to exhibit AI properties in vivo. In a study by Jagadish et al. (2016), in the carrageenan-induced acute inflammation test, petroleum ether fraction (SPEF) revealed a 39.16% AI effect (300 mg/kg of body weight orally), which was followed by ethyl acetate fraction (SEAF), alcohol fraction (SAF), and alcoholic extract with respective AI effects of 10.0, 22.5, and 5.0%. In the adjuvant-induced chronic inflammation test, the diclofenac extract and SPEF both significantly reduced inflammation (5 and 100 mg/kg of body weight orally for 7 days) [55]. Somchit and Shukriyah (2003) [31] reported the AI characteristics of intraperitoneally-administered aqueous and ethanol extracts of *Zz* at dosages of 25 to 100 mg/kg in prostaglandin-E2-(PGE2-)-induced paw edema test.

Zakaria et al. (2010) [56] showed that carrageenan-induced paw edema and cotton pellet-induced granuloma were treated with 100 mg/kg ASA (acetylsalicylic acid). In the paw edema test, 100 mg/kg ASA had a strong AI activity that was equivalent to that of 100 mg/kg methanol extract of *Z. zerumbet* (MEZZ), and in the granuloma test, it was comparable to that of 50 and 100 mg/kg MEZZ. In the paw edema test, ASA also showed a strong AI effect equivalent to that of 30, 100, and 300 mg/kg essential oil of *Z. zerumbet* (EOZZ) in the first three hours following administration. However, this activity dramatically decreased during the next two hours, while during this period, the activity of 100 and 300 mg/kg EOZZ was noticeably increased. Also, 100 mg/kg ASA generated around 60% anti-transudative and anti-proliferative effects in the granuloma test.

Conclusion

By evaluating the biological function of the selected ginger extracts in different polar and non-polar solvents, the study results revealed that the acetone extract of *Ac* demonstrated the lowest IC_{50} values in both DPPH (83.31 µg/mL) and hydrogen peroxide (85.43 µg/mL) scavenging assays; in addition, its DMSO extract showed the lowest IC_{50} value (89.59 µg/mL) in ABTS scavenging assay. By assessing the AI prospect, the study found that the petroleum ether extract of Zz had a lower IC_{50} value than the other solvents and gingers for inhibiting albumin denaturation (90.95 µg/mL) and RBC membrane hemolysis (HRBS assay) (83.53 µg/mL). The petroleum ether extract of *Ac* with an IC_{50} value of 82.56 µg/mL had the highest inhibitory activity against proteinases. The study identified specific solvent extracts of *Ac* (acetone, ethanol, and dimethyl sulfoxide) and *Zz* (petroleum ether) with potent antioxidant and anti-inflammatory activities, these extracts showed lower IC_{50} values in various assays, which indicate their effectiveness in scavenging free radicals (DPPH, hydrogen peroxide), inhibiting proteinases, and stabilizing cellular components (HRBS assay). These findings show that various gingers (*Ac* and *Zz*) exhibit varying degrees of bioactivity, with certain extracts consistently showing superior results across multiple assays. This specificity guide future research towards exploring unique chemical compositions and pharmacological potentials inherent in each species, these results highlight the suitability of *Ac* and *Zz* extracts as natural antioxidants and anti-inflammatory agents and provide important insights into the pharmacological potential of these extracts. The results provide a foundation for advancing research on natural product chemistry and therapeutic applications aimed at addressing health challenges associated with oxidative stress and inflammation. In addition, the results indicate that ginger plants possess antimicrobial qualities that

may find application in novel pharmaceutical formulations intended to treat bacterial infections.

Limitations of the study: Although the anti-inflammatory and antioxidant effects of ginger hold significant potential, some of the challenges in this research area include compositional unpredictability, bioavailability concerns, and the complexity of biological mechanisms.

Future research direction: Future research for exploring ginger anti-inflammatory and antioxidant capabilities could include investigating metabolic pathways, finding metabolites of ginger components, and better understanding their bioactivity and mechanisms of action in the body. In addition, large-scale, randomized, placebo-controlled, clinical trials are recommended to be performed to evaluate ginger efficacy and safety in a variety of inflammatory and oxidative stress disorders.

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Authors' contributions: RLR collected the samples, designed and analyzed the study, edited, drafted, and reviewed the manuscript thoroughly. JR supervised the study, read, edited, and revised the manuscript.

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