Trop J Nat Prod Res, February 2023; 7(2):2371-2381 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Prospects for Pharmaceutical of *Zingiber officinale* Extracts and Fractions by Analysis of the Chemical Markers, Safety Profile, Anti-Lipoxygenase, and Analgesic Activity

Zinna Marie P. Rasonabe*, Charlene G. Tiausas, Jannelle D. Cruz, Francheska Areza

Synnovate Pharma Corporation, Unilab Pharma Campus, 4024 Biñan, Laguna, Philippines.

ARTICLE INFO

Article history:
Received 06 January 2023
Revised 28 January 2023
Accepted 02 February 2023
Published online 01 March 2023

Copyright: © 2023 Rasonabe *et al*. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

Ginger (*Zingiber officinale*) has gained recognition for its flavoring uses and its numerous health benefits. Ginger can be used in functional foods and nutraceuticals alone or combined with other herbal plants. This study aims to validate the pharmaceutical potential of the *Zingiber officinale* variety from the Philippines by examining the chemical composition, acute oral toxicity, anti-lipoxygenase, and analgesic activities of its extracts and fractions. 6, 8, 10-Gingerols and 6, 8, 10-Shogaols were identified by high-performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) in methanol and hexane extracts. Acute oral toxicity in mice indicated the median lethal dose (LD_{50}) of methanol and hexane extracts of *Zingiber officinale*, and the 1% IPA-hexane and 50% IPA-hexane fractions obtained by solid-phase extraction (SPE) was greater than 2000 mg/kg body weight. The 1% IPA-hexane fraction altered the behavioral pattern of the animals. The anti-inflammatory activity was analyzed by lipoxygenase (LOX) inhibition assay. The IC_{50} value for the inhibitory action of crude methanol extract on LOX was 218.00 µg/mL. The crude hexane extract IC_{50} value was 57.40 µg/mL. SPE fractionation of hexane extracts resulted in higher IC_{50} values; 72.14 µg/mL for the 1% IPA-hexane fraction and 98.65 µg/mL for the 50% IPA-hexane fraction. Through *in-vitro* suppression of LOX, the hexane crude extracts showed more effective anti-inflammatory properties. The analgesic effect was measured using acetic acid-induced writhing in mice. All doses of the methanol and hexane extract inhibited writhing by a percentage ranging from 91-100%. The SPE fractions of the hexane extract have 78-99% inhibition. Both extracts and fractions demonstrated strong analgesic effects in the mice models.

Keywords: *Zingiber officinale*, High-performance liquid chromatography, Liquid chromatography-tandem mass spectrometry, Acute oral toxicity, Anti-inflammatory, Analgesic

Introduction

Ginger, *Zingiber officinale* (ZO), a member of the Zingiber genus in the Zingiberaceae family, is one of the most significant and well-known traditional herbs, having been used for years as a valuable spice and traditional medicine.[1] It is widespread throughout the tropical regions of Asia, Africa, America, and Australia.[2] It has been noted for its distinctive smell and pungent flavor.[3]

Ginger's rhizome contains diverse bioactive compounds, including phenolic and terpenes, and has health-promoting benefits.[4] The different bioactivities of ginger can be attributed to gingerols, shogaols, and paradols, the predominant phenolic compounds.[5] Several terpene compounds, including *β*-bisabolene, *α*-curcumene, zingiberene, *α*-farnesene, and *β*-sesquiphellandrene, are considered the primary components of ginger essential oils.[6] Ginger exhibited various biological functions, such as antioxidant, anti-inflammatory, anticancer, neuroprotective, respiratory protective, antiobesity, antidiabetic, antinausea, blood pressure-lowering, cholesterol-lowering, antimicrobial, antiplatelet aggregation, and activities.[4,7]

Corresponding author. E mail: zprasonabe@unilab.com.ph
Tel: +639153131677

Citation: Rasonabe ZMP, Tiausas CG, Cruz JD, Areza F. Prospects for Pharmaceutical of *Zingiber officinale* Extracts and Fractions by Analysis of the Chemical Markers, Safety Profile, Anti-Lipoxygenase, and Analgesic Activity. Trop J Nat Prod Res. 2023; 7(2):2371-2381 http://.www.doi.org/10.26538/tjnpr/v7i2.11

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

It's significant to mention that ginger has gained popularity in the Philippines, not just because of its flavor uses but also because of its numerous health advantages. A growing number of functional foods and nutraceuticals now contain ginger, either alone or in combination with other herbal plants, because of its potential to improve health. The effects of adding 2% inclusions of ginger, black pepper, garlic, turmeric, and a mixture of these spices on serum lipid profiles, atherogenic indices, and indicators of liver function were examined in a study on rats with the induced-metabolic syndrome. Low-density lipoprotein cholesterol, triglyceride, and serum cholesterol levels all decreased significantly. The activities of aspartate aminotransaminase, alanine aminotransaminase, alkaline phosphatase, and gamma-glutamyl aminotransaminase were all significantly lowered, as were the atherogenic index and coronary risk index. The results showed that individually and in combination, dietary additions of ginger, black pepper, garlic, and turmeric powder improved dyslipidemia and serum enzyme activity. [8]

In a recent focused review, the anti-inflammatory and analgesic properties of ZO preparations were underlined, along with an expanding discussion of the experimental pharmacology and clinical efficacy. In summary, there is a clear indication from various preclinical studies that ginger may have anti-inflammatory and analgesic properties. In inflammation, the molecular mechanism among ginger constituents, 10-gingeridone, 6-shogaol, 6-gingerol, and zingerone, mainly affects the NF-kB signaling pathway, which involves the reduction of NF-kB-related gene expression (i.e., cytokines, iNOS, COX2).[9] Ginger extract and 6-shogaol have also been shown to reduce hyperalgesia and allodynia in a mouse model of painful diabetic neuropathy. This effect is mediated by decreased expressions of Transient receptor-potential cation channel- subfamily V member 1 (TRPV1) and N-methyl-D-

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

aspartate receptor subunit 2B (NMDAR2B) in the spinal cord.[10] Ginger's clinical efficacy has also been demonstrated in various inflammatory and painful conditions, including osteoarthritis, migraine, and dysmenorrhea.[9]

Despite the accumulation of research, experimental validations of the Philippine ginger variety have yet to be exploited for their effective use in inflammation-associated and pain conditions. Nevertheless, local communities in the Philippines utilize ginger rhizome to treat body pain and sprain,[11] alleviate stomachache and rheumatism,[12] relieve sore throat.[13] Research findings support the long-held claim that ginger has phytochemicals with anti-inflammatory and analgesic activities. Therefore, the present study aims to establish the chemical markers of the extracts and fractions of ZO from the Philippines using chromatographic and mass spectrometric analytical methods, followed by an investigation of their potential anti-lipoxygenase and analgesic action by *in-vitro* and *in-vivo* experiments.

Materials and Methods

Plant materials

A local ginger (*Zingiber officinale)* rhizome was supplied from a farm in Barangay Dumarao Roxas, Palawan, Philippines, a partner of Palawan Center for Appropriate Rural Technology, Inc. (PCART), under the registered company named Palawan Bio-Farm Enterprises. The plant was verified by the Philippines' Department of Agriculture's Bureau of Plant Industry. The raw materials processing procedures were completed at the PCART facility and were collected in February 2020 with batch number 2003796. The process is as follows: when the plant's leaves have dried, the rhizome is harvested in the morning. The farmers were provided with new plastic bags to store their harvested leaves. Next, the harvested materials are transported to the facility's garbling and washing sections. Within two hours of harvesting, the drying process is complete. The moisture content of dried leaves is between 7-8 %. After the leaves have been dried and cooled, they are placed in bags made of food-grade polyethylene, sealed, and transported to the processing facility. It was ozonated, pulverized to mesh size 10, and finally dried to a maximum moisture content of 5 % in an industrial oven.

Chemicals

HPLC-grade acetonitrile, methanol, hexane, 2-propanol (IPA), and other analytical-grade reagents were obtained from Theo-Pam Trading, Philippines. The other chemicals used were purchased from Belman Laboratories, Philippines. 1-Dehydro-10-gingerdione, 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, 8-shogaol, and 10-shogaol standards were acquired from China's Chem Faces Biochemical Co., Ltd. Glycine max (soybean), Lipoxygenase (Catalog Number: L7395), Linoleic acid (Catalog Number: L1376), and Indomethacin (Catalog Number: I8280) were purchased from Sigma-Aldrich for the LOX assay.

Preparation of standards

Stock solutions of the reference compounds in methanol, 1-dehydro-10-gingerdione, 6-, 8-, and 10-gingerols, 6-, 8-, and 10-shogaols, were all prepared individually. Before use, all of the standard solutions were kept at 4°C. The final concentration of the mixed standard solution was prepared by taking an appropriate volume of each stock standard. The mixed standard solution was ultrasonically filtered through a 0.20 m PTFE membrane filter and then subjected to analysis. The samples of ZO leaves were then examined using HPLC and standard reference compounds.

Preparation of plant extracts

Zingiber officinale (ZO) powdered leaves were extracted by an ultrasonic-assisted extraction (MRC Scientific Instruments Professional Ultrasonic, UK) set at 40 kHz, 30°C for 60 minutes. The solvents individually used were 100% methanol and 100% hexane (25 g powder leaves in 200 mL solvent). Following extraction, the mixture was filtered through cheesecloth and filter paper, concentrated with a rotary evaporator (IKA, Germany), and stored at 4°C for up to a month in an amber bottle. For every subsequent experiment, a fresh extract solution was prepared.

Solid-Phase Extraction (SPE) fractionation of the extracts

SPE was used to fractionate a 25 mL of 20 mg/mL hexane extract solution. The strata Si-1 (normal phase, 1 g/6 mL) cartridge was attached to a Phenomenex SPE vacuum manifold, conditioned with 100% IPA, then equilibrated with 100% hexane. The extract solution was subsequently poured gently through the preconditioned cartridge. Two solvents were used to elute the SPE column, 1% IPA-hexane (ZO Hexane SPE fraction 1) and 50% IPA-hexane (ZO Hexane SPE fraction 2). Each collected fraction was concentrated using rotary evaporators operating at 80 rpm, 40°C water baths, and 10°C chillers. The fractions underwent HPLC analysis and biological activity tests.

High-Performance Liquid Chromatography (HPLC)

Zingiber officinale (ZO) extract dissolved in methanol (10 mg/mL) and hexane (5 mg/mL) was passed separately through a 0.2 µm PTFE membrane filter to obtain a clean solution. Then, each extract and the standard cocktail were independently analyzed to HPLC. The HPLC system was comprised of a separation module (Shimadzu Prominence) outfitted with LabSolutions software (Shimadzu) and a binary pump, needle-in-flow path autosampler, and a photodiode array (SPD-M20A) detector. The analysis was conducted on a Waters Xbridge C18 (4.6250 mm, 5.0 µm) column employing gradient elution with 0.5% phosphoric acid (solvent A) and acetonitrile (solvent B) as the mobile phase. Every mobile phase solvent was filtered with a 0.45 µm membrane filter. 20 µL of standard and sample mixtures were introduced into the HPLC and separated at a flow rate of 0.5 mL/min. The column oven temperature was at 30°C, and the HPLC peaks were observed on the UV detector at 254 nm. The gradient elution used was: 0-10 min, 85-60% A and 15-40% B; 10-30 min, 60-45% A and 40-55% B; 30-40 min, 45-25% A and 55-75% B; 40-65min, 25-20% A and 75-80% B; 65-80 min, 20-5% A and 80-95% B; 80-90 min, 5-85% A and 95-15% B. The column was washed and equilibrated before the next injection.

Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS)

Extracts and their HPLC peak fractions were further assessed using an ESI-QTOF-MS/MS system comprised of a Waters ACQUITY I-Class UPLC and a Waters Xevo G2-S QTOF mass spectrometer. Two µL samples were separated on Acquity HSS C18 column (waters, 2.1×100 mm, 1.8 µm) reverse-phase at 30 °C and gradient-eluted at a flow rate of 0.25 mL/min. The mobile phase was 0.1% formic acid-water (solvent A) and 0.1% formic acid-acetonitrile (solvent B). The HPLC gradient method was converted to a UPLC method using columns calculator version 2.0.53.0 (Waters Corporation) to match the LC-MS system with little optimization. Data was controlled with Mass lynx 4.1. The data range of 100-1500 Da, applied source temperature of 20°C, desolvation temperature of 450°C, cone gas flow rate of 50 L/h, desolvation gas flow rate of 600 L/h, Electrospray ionization, positive mode capillary voltage of 3.0 kV, and cone voltage of 80 V was the acquisition settings. MSE mode, low collision energy scan of 6 eV, high collision energy scan of 30 to 50 eV, the scan time of 0.1 s. The MS-DIAL software converted the output RAW files to ABF for peak alignment, peak picking, and identification. The following libraries are used by the spectral databases: Faulkner Legacy, NIH Natural Products, Prestwick Phytochemicals, GNPS, Sumner, ReSpect, MassBank EU, MassBank NA, and Dorrestein/FDA Natural Products. The spectral databases include libraries such as GNPS, Sumner, ReSpect, MassBank EU, NIH Natural Products, Prestwick Phytochemicals, Dorrestein or FDA Natural Products, MassBank NA, and Faulkner Legacy. Several MS-DIAL-processed data were further processed in GNPS to match compounds.

Lipoxygenase (LOX) inhibitory activity

25 µL of extracts or SPE fractions and 100 µL of lipoxygenase solution (in borate buffer pH 9.0) at a concentration of 0.0045 mg/mL were mixed and incubated in a 96-well quartz plate for 5 minutes at room temperature. Then, 25 µL of 0.84 mg/mL linoleic acid was added and adequately mixed. The absorbance at 234 nm was measured with a microplate reader (Biotek Synergy HT). As a sample blank, borate buffer was employed, and DMSO-borate buffer (enzyme + substrate only) was used as a control. The % inhibition was calculated by using the following:

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

% Inhibition = [$A_{control} - A_{test\ group} / A_{control}$ x 100, A= Mean absorbance. The drug Indomethacin served as a positive control. The IC_{50} values were determined using a dose-response curve. The IC_{50} represents the concentration necessary to achieve 50% of the maximum inhibitory capacity.

Experimental animals

The animals were kept in a room with a constant temperature of 22±3°C, relative humidity of 50-60%, a light-dark cycle of 12 h: 12 h, an unlimited supply of sterile water, and a regular laboratory food (Sarimanok, Unahco). The animals were allowed seven days to adjust to laboratory surroundings before being used in experiments. The study complied with Philippine laws governing animal welfare. The Institutional Animal Care and Use Committee (numbers IACUC-BSD-001-29-01, IACUC-019-014) and the Bureau of Animal Industry examined and approved the procedure (reference numbers AR-2020-085, AR-2020-083).

Acute oral toxicity

The acute oral toxicity of *Zingiber officinale* (ZO) extract and SPE fractions was evaluated using the "Up-and-Down" method outlined in OECD Guideline 425 (US Environmental Protection Agency, 2003; OECD, 2008). Swiss Webster mice (healthy, nulliparous, non-pregnant, 8-12 weeks old, weighing 20-25 grams) were used for testing. They were given water but fasted for three to four hours before receiving the treatment. The test samples were given orally in a single dose using an oral gavage tube. The study utilized a limit test at 2000 mg/kg. The main test at 175, 550, and 2000 mg/kg was conducted if test samples showed death in the limit test after the first animal dose. Following treatment, the mice's general behavior and clinical indicators of toxicity were observed continuously for the first hour and then every 30 minutes for the next six hours. Each animal was observed for adverse effects at 24 hours and 14 days. The experimental measures include salivation, diarrhea, lethargy, convulsions, eyes, mucous membranes, respiratory and circulatory systems, and changes in the skin, fur, and other body parts.

Analgesic activity using acetic acid-induced writhing in mice

The analgesic action was tested using Swiss Webster female mice (healthy, nulliparous, 8-12 weeks old, and weighing 25-30 grams). Animals were given water but fasted for three to four hours before receiving the medication. Mice that received intraperitoneal injections of 0.6% acetic acid (10 mL/kg body weight) exhibited writhing. In the peritoneal region, a painful reaction and acute inflammation emerge, creating reflexive writhing. After preliminary screening, only animals who writhed within 15 minutes were included in the study. The animals were allowed 48 hours of relaxation. The pre-screened animals were randomly allocated into six groups. The test medications were administered orally to the mice 30 minutes before the acetic acid injection. Immediately following injection, individual mice were placed in a glass viewing jar, and five minutes elapsed. During the next twenty minutes of observation, the number and duration of each animal's writhing were recorded. The percentage of inhibition of writhing was assessed as an index of analgesia.

% Inhibition = $NW_{Control} - NW_{test\ group} / NW_{Control}$ NW = Mean number of writhes

Statistical analysis

The results were presented as mean (±) standard deviation (SD) or standard error of the mean (SEM). Analysis of Variance (ANOVA) or Kruskal-Wallis was used for statistical analysis in the SPSS statistics software (version 27). It was regarded statistically significant if $P < 0.05$.

Results and Discussion

Chemical Markers of Zingiber officinale (ZO) Extracts

Zingiber officinale extracts were examined using high-pressure liquid HPLC to determine the chemical components present. In this method, multicomponent extracts are separated and identified relative to one another. As depicted in Figures 1B and 1C, the HPLC chromatograms of methanol and hexane extracts, respectively, were found to be almost comparable. Although, additional minor peaks were seen between 13-25 min in methanol extract, and a higher peak intensity eluted in the range of 42-67 min in hexane extract. As presented in Figure 1A, six reference standards for ZO were analyzed under the same chromatographic system of ZO extracts. Comparing the RT of peaks with that of the injected standards, six peaks at 34.7, 45.9, 47.9, 53.7, 55.6, and 67.0 min were identified as 6-Gingerol (1), 8-Gingerol (2), 6-Shogaol (3), 10-Gingerol (4), 8-Shogaol (5), and 10-Shogaol (6), respectively, both in methanol and hexane extracts. The peak of 6-Shogaol was higher in hexane extract than in methanol. Lantz et al. (2007) detected predominant compounds in the dichloromethane-methanol (1:1 v/v) extract of ZO: 6-, 8- 10-gingerols and 6-, 8-, 10-shogaols. Fractions that contain mainly gingerols or shogaols strongly inhibited lipopolysaccharide (LPS)- induced prostaglandin E2 (PGE2) production of U937 cells. A combination of compounds was more efficient than each tested component alone.[14] These detected peaks in both extracts are probable bioactive compounds. Future research should examine the volatile components of the hexane extracts that might be pharmacologically active. Linalool, borneol, zingiberene, and zingerone were found to be the primary components of the white ginger (*Zingiber officinale Roscoe*), which was extracted using a soxhlet extractor and hexane solvent.[15]

A previous study investigated Zingerone's therapeutic effects in a carrageenan-induced inflammatory model. Using various doses of the chemical standard Zingerone (10, 20, and 40 mg/kg) considerably reduced paw edema in carrageenan-injected rats, with the highest dose of 40 mg/kg being the most effective. Additionally, it lowered paw levels of MDA, NO, COX-2, PGE_2, TNF-α, and IL-1β.[16] The levels of inflammatory markers such as TNF-α, IL-6, NF-κB, and IL-1β in rats with isoproterenol-induced myocardial infarction were considerably lowered by zingiberene pre-treatment.[17]

LC-MS/MS untargeted analysis was performed to obtain information about the molecular mass and further identify the *Zingiber officinale* (ZO) extract's chemical composition. For MS identification, the positive-ion mode was used for acquiring mass spectra from the UPLC-QTOF-MS/MS analysis. Plant components were analyzed from crude extracts or HPLC fractions. Figure 2 represents the chromatograms with MS and UV detectors of methanol (2A) and hexane (2B) ZO extracts. The majority of compounds were detectable within 13 min. The chromatographic and mass spectrum characteristics of the detected peaks are displayed in Table 1. The predominant ion in the positive ion mass spectra of Gingerols is the [M+Na], whereas the principal ion in the positive ion mass spectra of Shogaols is the [M+H]. The compounds found in the chromatograms were determined based on mass spectral data, comparing retention periods with standard reference compounds and mass spectra with those previously published.[18,19,20] As indicated in Table 1, major secondary metabolites characterized and identified from ZO extracts or HPLC fractions (data not shown) are 6-Gingerol (1), 8-Gingerol (2), 6-Shogaol (3), 10-Gingerol (4), 8-Gingerol (5), and 10-Shogaol (6). However, several peaks need further elucidation and characterization because, in the study, MS-DIAL and GNPS analysis did not match them. Therefore, additional reference standards, mass libraries, and spectroscopy methods will be useful.

Chemical Profile of Zingiber officinale (ZO) Fractions

Solid-phase extraction (SPE) is increasingly recognized as a technique for separating and concentrating components from liquid samples. ZO hexane extract obtained via ultrasonic-assisted extraction was subjected to SPE to remove matrix components, fractionate, and separate the chemicals in each extract according to their polarity. SPE in the normal phase was applied for the fractionation of hexane extract. Two distinct solvent ratios were eluted in the SPE column: 1% IPA- hexane (ZO Hexane SPE fraction 1) and 50% IPA- hexane (ZO Hexane SPE fraction 2). Figure 3 compares the HPLC chemical profiles of the ZO crude extract and corresponding SPE fractions. During SPE fractionation, the non-polar components present in the hexane extract (Figure 3A) were separated. As a result, 1% IPA- hexane fraction 1 (Figure 3B) contains compounds soluble in the more non-polar solvent, including 6-Shogaol (3), 8- Shogaol (5), and 10-Shogaol (6). On the other hand, 50% IPA-hexane fraction 2 (Figure 3C) has the less non-polar part showing 6-

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Gingerol (1), 8-Gingerol (2), and 10-Gingerol (4). Gingerols are aromatic phenolic structures consisting of a series of structural analogs of 1-(3-methoxy-4-hydroxyphenyl) 3-oxo-5-hydroxy-hexane with varying lengths of the unbranched alkyl side chain. [21] The 4-, 6-, 7-, 8-, 10-, and 12- gingerol are its derivatives, and the length of its unbranched alkyl side chain distinguishes gingerols. [22] Conversely, shogaol can be formed from gingerols by removing the C5 hydroxyl and forming a C4 and C5 double bond. [21]

ZO extracts and SPE fractions were subjected to *in-vitro* and *in-vivo* bioassays to compare their activities and infer constituents involved in plant activity.

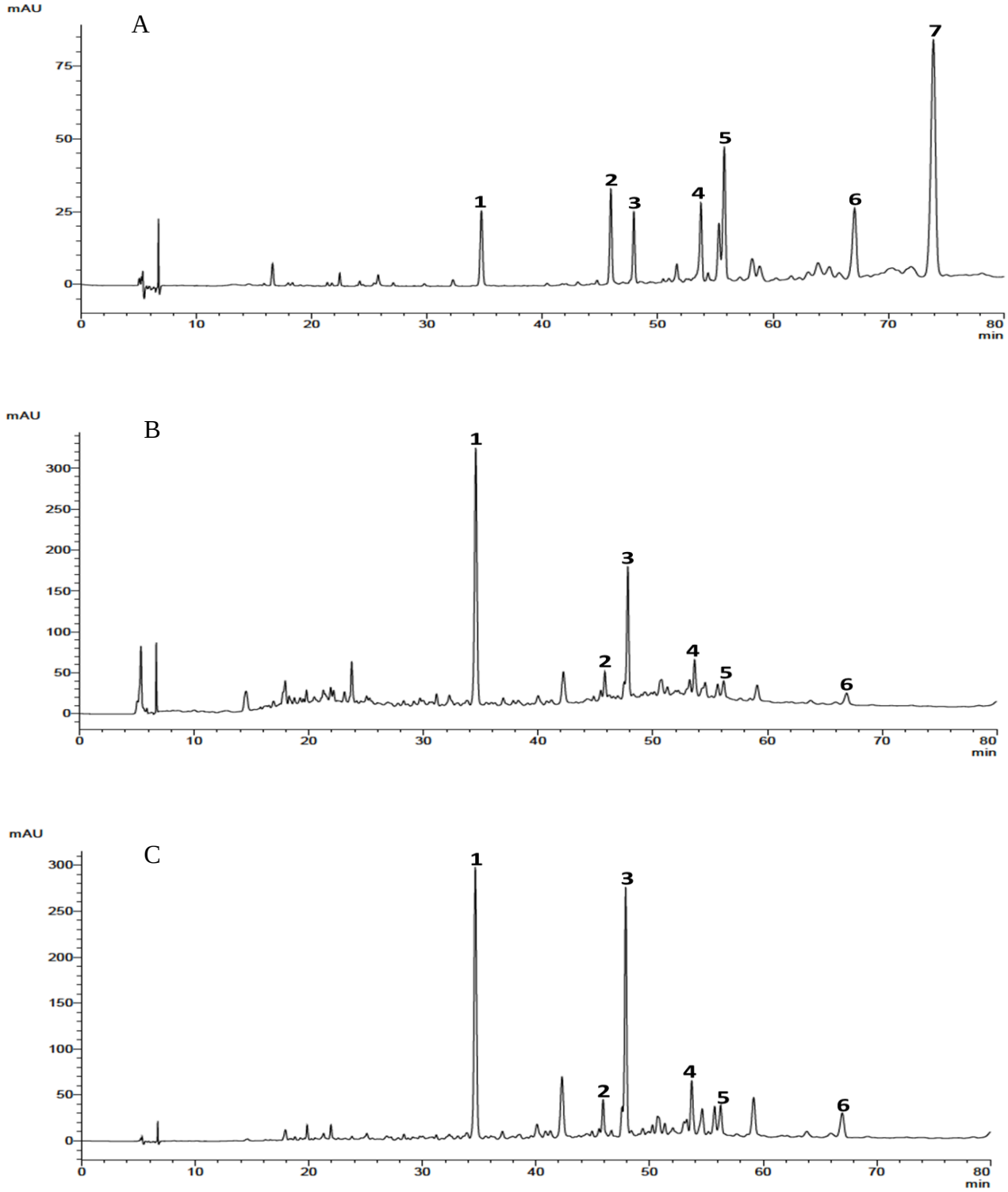

Figure 1: HPLC Chromatograms of A. Standard compounds6-Gingerol (1), 8-Gingerol (2), 6-Shogaol (3), 10-Gingerol (4), 8-Shogaol (5), 10-Shogaol (6), 1-Dehydro-10-gingerdione (7), B. methanol, and C. hexane extracts of *Zingiber officinale*

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

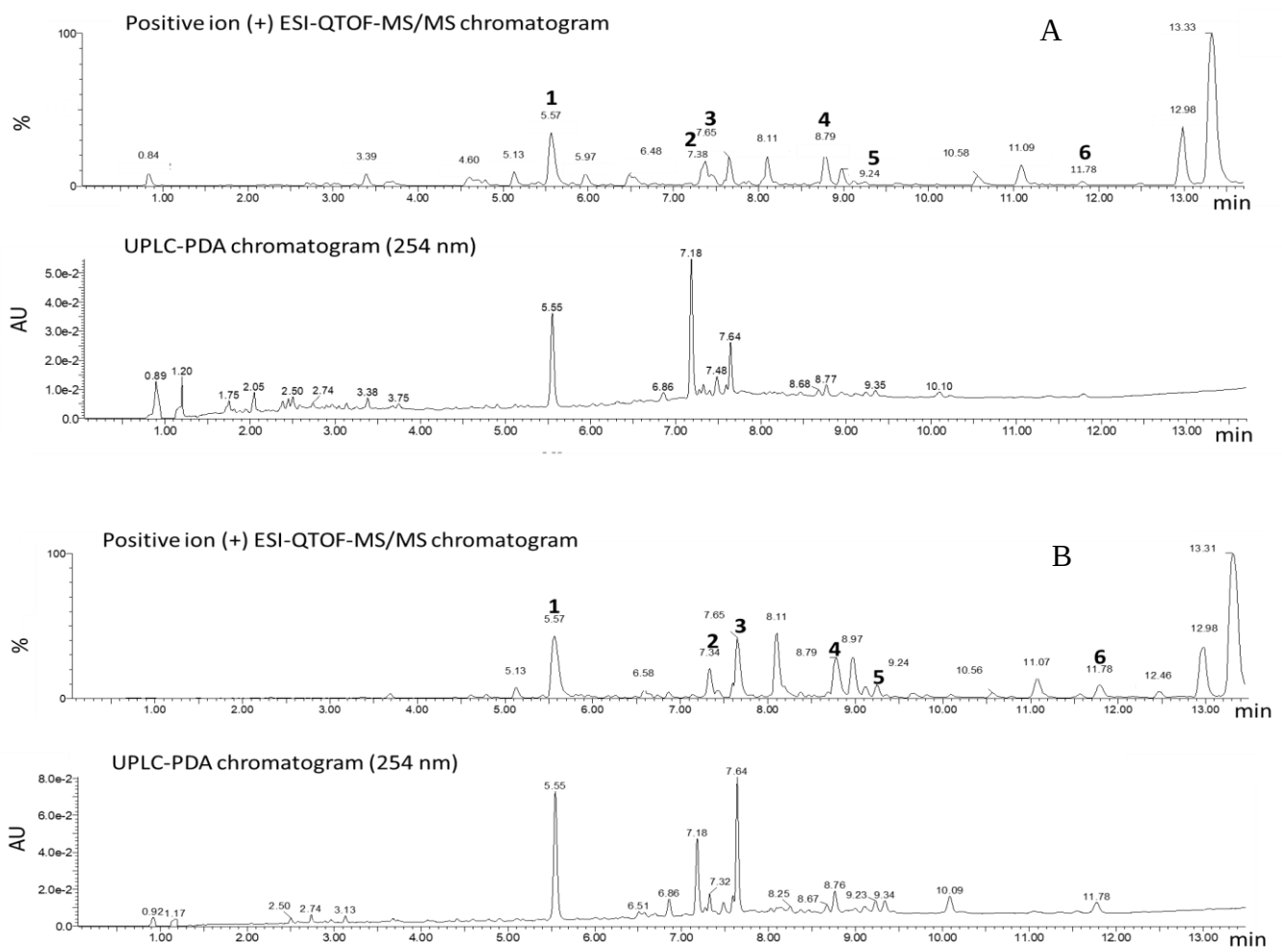

Figure 2: LC-MS/MS analysis of A. methanol and B. hexane extracts of *Zingiber officinale* showing the positive ion (+) ESI-QTOF-MS/MS and UPLC-PDA chromatograms

Anti-Inflammatory activity through lipoxygenase (LOX) inhibition

The effect of *Zingiber officinale* (ZO) on lipoxygenase (LOX) enzyme activity was investigated. This enzyme participates in the LOX pathway of arachidonic acid metabolism, generating pro-inflammatory mediators such as leukotrienes (LT). This pathway's products are linked to the onset of inflammatory-related diseases. The assay was conducted to determine the conversion of linoleic acid to linoleic hydroperoxide in the presence of LOX. The progression of the LOX-catalyzed reaction would be hindered by treatment with an enzyme inhibitor. Table 2 illustrates the effect of methanol and hexane extracts of ZO and fractions obtained by solid-phase extraction (SPE) on a dose-dependent inhibition of LOX activity. Test samples were tested at eight different concentrations together with the standard Indomethacin. The percentage inhibition of enzyme activity and IC_{50} values were used to express the inhibitory effect of the compounds tested. Lower IC_{50} values are indicative of higher inhibitory activity. In Table 2A, the methanol extract of ZO has low inhibitory activity with IC_{50} = 218.00 µg/mL. On the other hand, its hexane extract revealed a high LOX inhibition effect with IC_{50} value = 57.40 µg/mL. The HPLC profiles of hexane and methanol extracts are almost identical, although hexane exhibits higher peak intensity of specific metabolites like 6-Shogaol, which may account for their activity differences. Recent research has shown that 6-Gingerol and 6-Shogaol may be effective anti-inflammatory agents. The in-silico method determined that these two active compounds in ZO inhibit 5-LOX due to their specific interactions with residues involved in molecular inhibition.[23] Research also suggested that the primary ginger components, 6-gingerol, and 6-shogaol, are responsible for the diverse effects. Furthermore, it has been found that, in many cases, the bioactivity of shogaols is more potent than gingerols. [24,25] 6-shogaol is the dehydrated form of 6-gingerol, and during thermal processing, shogaols are generated from the corresponding gingerol.[26] The IC_{50} for the ZO hexane extract is lower than 1% IPA- hexane (ZO Hexane SPE fraction 1) and 50% IPA- hexane (ZO Hexane SPE fraction 2). As shown in Table 2B, fraction 1 has an IC_{50} of 72.14 µg/mL, equivalent to Indomethacin's IC_{50} of 73.69 µg/mL. The IC_{50} value for Fraction 2 is 98.65 µg/mL, slightly higher than fraction 1. Fraction 1 contains the Shogaols (1% IPA-hexane), whereas fraction 2 has the Gingerols (50% IPA-hexane). Multiple investigations show evidence that 6-shogaol can be classed as an anti-inflammatory agent. Commonly recognized indicators and signaling pathways of inflammation were successfully inhibited when 6-shogaol was applied to several cell types or in vivo models. Inhibiting NFκB or COX-2, attenuating iNOS levels, resulting in lower NO levels, and attenuating the release of pro-inflammatory cytokines like interferon, TNF, interleukins, and chemokines are just a few of the pro-inflammatory agents and mediators that 6-shogaol suppressed. Additionally, 6-shogaol stimulated protective effects by elevating Nrf2 levels, leading to an increased amount of HO-1 protein. [27] The SPE fractionation produced lesser active fractions than the crude hexane extract. Therefore, further purifying ZO extracts may not be necessary for LOX enzyme inhibition. During the fractionation process, various active components important to its action were separated, such as the gingerols and shogaols. Gingerols, Shogaols, and other structurally-related components in ginger, such as Gingerdione, Capsaicin, and Diarylhepatanoids, were active against 5-lipoxygenase, an enzyme involved in leukotriene (LT) biosynthesis.[28]

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

Acute oral toxicity

To provide preliminary results on the toxicity level of hexane and methanol extracts of *Zingiber officinale* (ZO) and fractions derived from SPE, acute oral toxicity (AOT) tests were carried out adopting OECD No. 425 acute oral toxicity: Up-and-Down-procedure. The fractions include 1% IPA- hexane (ZO Hexane SPE fraction 1) and 50% IPA- hexane (ZO Hexane SPE fraction 2), derived from its hexane extract. Initially, a limit test at 2000 mg/kg body weight was conducted with one animal from each treatment sample, vehicle, and negative control group. Four additional animals were sequentially dosed when the first animal survived, so five were tested. However, main tests were conducted for test samples with reported death.

Test animals were observed for their behavior in the first 30 min or 1 h, 6 h, and then for 24 h. The observations were recorded over 14 days. In Table 3A, the behavioral pattern showed decreased activity and respiratory changes after dosing with hexane and methanol extracts of ZO in the first 30 min. However, it was recorded as normal in the next time intervals. Animals were initially given 1% IPA- hexane (ZO Hexane SPE fraction 1) at 2000 mg/kg body weight and showed hypoactivity with respiratory changes before observing death within 24 hours. Therefore, the sample proceeded with the main test. A total of five animals were sequentially dosed at 175, 550, and 2000 mg/kg body weight. All of the animals survived. However, after receiving a dose of 2000 mg/kg body weight, some developed itching, salivation, hypoactivity, and respiratory changes within 6 hours. On the other hand, 50% IPA- hexane (ZO Hexane SPE fraction 2) treatment in animals has survived without observed toxic manifestations. Table 3B showed that animals given treatment with ZO methanol and hexane extracts, hexane SPE fractions, vehicle controls, and distilled water survived. According to the findings, the median lethal dose (LD_{50}) of methanol and hexane extracts of ZO and its SPE fractions was greater than 2000 mg/kg body weight. However, 1 % IPA- hexane (ZO Hexane SPE fraction 1) has changed the behavior pattern of the animals and thus is classified under Hazard Category 4 in the Globally Harmonized System (GHS). 1% IPA-hexane fraction includes the more potent bioactive 6-Shogaols. Ginger is widely regarded as a safe, natural remedy. [29] In contrast, ginger's harmful effects have been recorded on pregnant rats. [30] Ginger has been related to some slight negative effects in humans. One participant in the research experienced minor diarrhea during the initial two days of ginger pre-treatment. Ginger can cause heartburn and, in dosages greater than 6 g, can irritate the stomach. Inhalation of ginger dust may cause IGE-mediated allergies. [31] Metabolites 6-, 8-, 10-gingerols, and 6-shogaol were safe for healthy human volunteers at doses up to 2000 mg. Several people developed minor gastrointestinal symptoms at the highest doses, such as eructation, heartburn, and indigestion. [32]

Analgesic activity

The analgesic potential of methanol and hexane extracts of *Zingiber officinale* (ZO) and fractions derived by SPE of hexane extract was evaluated in mice using a writhing test induced by acetic acid. It is a chemical technique used to generate pain of peripheral origin in mice by injecting a specific quantity of acetic acid. The number of writhing and percentage of inhibition of the treated and untreated groups are shown in Table 4. Diclofenac treatment at 5 mg/kg completely prevented writhing in test animals, whereas distilled water and vehicle control PEG 400 had no significant impact. All treatment groups revealed a statistically significant ($p<0.05$) decrease in the number of writhes compared to the vehicle control groups. All doses of methanol and hexane ZO extracts inhibited writhing by a percentage ranging from 91% to 100%. The SPE fractions from hexane extract showed 78-99% inhibition. Only fraction 2 of ZO Hexane (ZO Hexane SPE fraction 2) displayed a dose-dependent inhibition writhing response. The level of inhibition did not differ significantly between crude hexane extracts and fractions, indicating that the analgesic effect of crude components and ZO fractions in animal testing may not be specific. The oral treatment of *Hypericum reflexum* infusions, methanol extracts, and various fractions (aqueous, butanol, and chloroform) on acetic acid-induced writhing reactions in mice dramatically reduced the number of abdominal constrictions, indicating analgesic efficacy. Since acetic acid acts indirectly by causing the release of endogenous mediators, which stimulate nociceptive neurons that are sensitive to nonsteroidal anti-inflammatory drugs, narcotics, and other centrally active drugs, it is believed that acetic acid-induced constriction is a nonselective antinociceptive model. [33] 6-gingerol inhibited acetic acid-induced writhing response and formalin-induced licking time in the late phase. It also reduced paw edema in carrageenan-injected animals. [34] The treatment of 6-gingerol and 6-shogaol reduced the writhing symptom and improved lowered pain threshold, as measured by the whittle and Randall-Selitto techniques, respectively. However, these effects of 6-Shogaol were more intensive. [35] ZO extracts derived from methanol and hexane include both gingerols and shogaols. 1% IPA- hexane (ZO Hexane SPE fraction 1) has 6-shogaols, whereas 50% IPA- hexane (ZO Hexane SPE fraction 2) contains 6-Gingerol. All extracts and fractions exhibited effective analgesic properties. This may suggest that these two compounds produce independent analgesic actions. Ginger's bioactive components include gingerols (23-25%), shogaols (18-25%), and related ketone derivatives. [22] There is still more research to be done to determine which shogaols and gingerols have the most significant pharmacological function.

Conclusion

HPLC and LC-MS/MS analysis identified several compounds, such as 6-Gingerol, 8-Gingerol, 6-Shogaol, 10-Gingerol, 8-Gingerol, and 10-Shogaol. 1% IPA-hexane fraction showed signs of toxicity in mice. ZO Extracts and fractions exhibited anti-inflammatory effects by inhibiting LOX enzymes and analgesic effects in an animal model. The LOX enzyme inhibition test showed that SPE fractionation did not enhance activity. The study showed scientific evidence that the leaves of Philippine *Zingiber officinale* (ZO) have anti-inflammatory and analgesic effects and thus can be exploited to produce medicinal products. Future investigations will focus on identifying and elucidating further active compounds responsible for both activities.

Table 1: Chromatographic and mass spectral properties of peaks identified from *Zingiber officinale* using LC-MS/MS analysis

No.	RT (min)	Precursor ion	Experimental mass	Fragments	Identification
1	5.57	[M+Na]	317.1875	277.18, 259.16, 177.09, 137.06	6-Gingerol
2	7.34,7.38	[M+Na]	345.1991	305.21, 287.20, 177.09, 137.06	8-Gingerol
3	7.65	[M+Na]	299.1568	275.16, 229.14, 137.06	6-Shogaol
4	8.79	[M+Na]	373.2312	333.24, 315.23, 177.09, 137.06	10-Gingerol
5	9.24	[M+H]	305.2061	301.14, 205.19, 137.06	8-shogaol
6	11.78	[M+H]	333.2374	229.14, 137.06	10-Shogaol

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

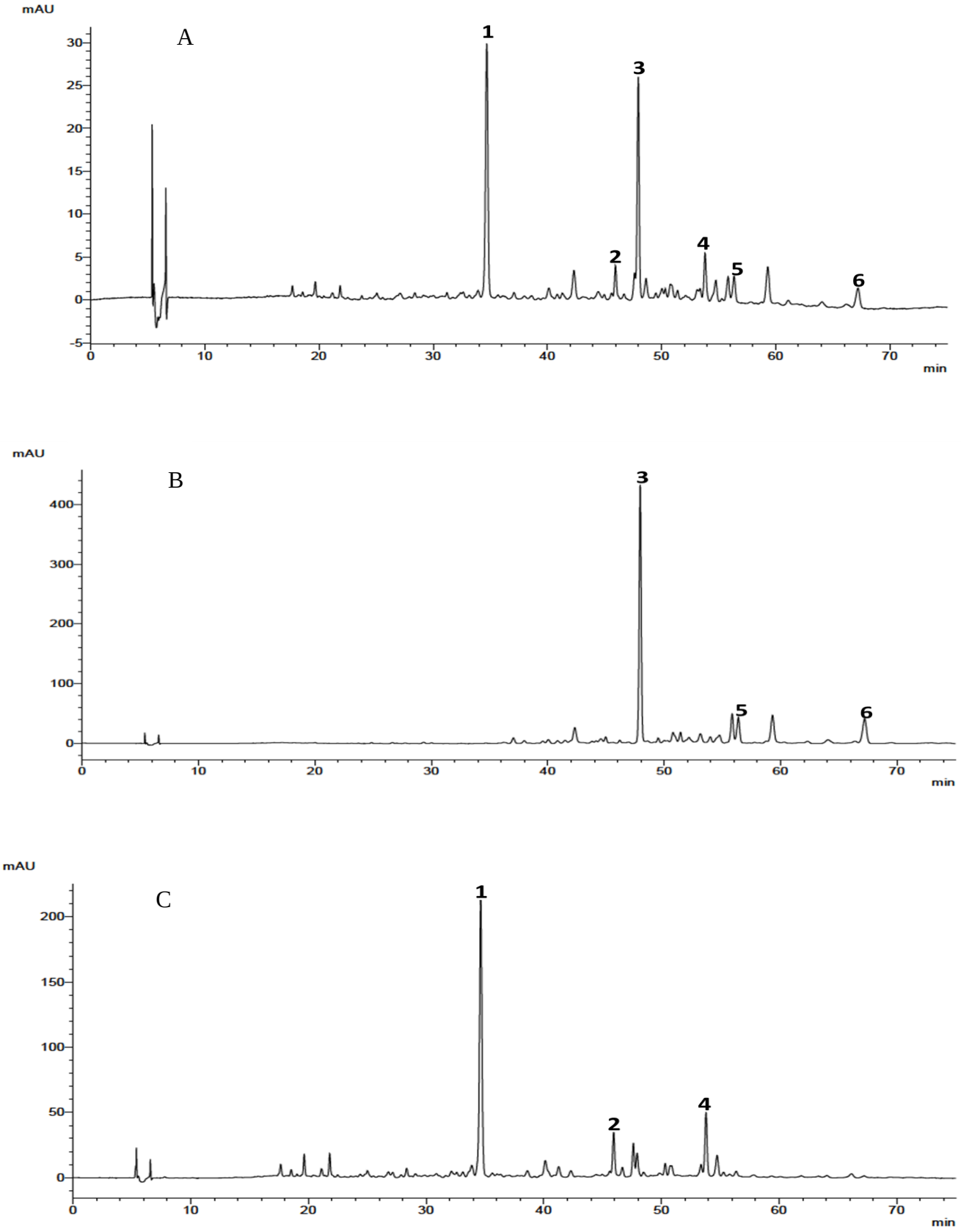

Figure 3: HPLC Chromatograms of A. hexane extract of *Zingiber officinale* showing 6-Gingerol (1), 8-Gingerol (2), 6-Shogaol (3), 10-Gingerol (4), 8- Shogaol (5), 10-Shogaol (6), B. ZO Hexane SPE fraction 1, C. ZO Hexane SPE fraction 2
ZO: *Zingiber officinale*, SPE: Solid-phase extraction

Table 2A: Lipoxygenase inhibitory activity of *Zingiber officinale* methanol and hexane extracts

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

ZO Methanol			ZO Hexane		
		IC_{50} (95% CI)			IC_{50} (95% CI)
mg/ml	% Inhibition	mg/ml	mg/ml	% Inhibition	mg/ml
50.0	3.82 ± 1.38	218.00	5.0	8.41 ± 3.21	57.40
100.0	17.80 ± 1.66	(212.50-223.50)	10.0	16.18 ± 3.52	(54.03-60.97)
200.0	43.34 ± 1.40		25.0	34.39 ± 4.10	
250.0	57.81 ± 1.68		50.0	47.99 ± 3.46	
300.0	66.26 ± 2.25		75.0	55.67 ± 2.73	
350.0	79.27 ± 1.86		150.0	75.96 ± 3.57	
400.0	86.08 ± 1.98		300.0	93.77 ± 3.04	
500.0	96.11 ± 1.12		500.0	96.27 ± 2.69	

Table 2B: Lipoxygenase inhibitory activity of *Zingiber officinale* SPE fractions

ZO Hexane SPE fraction 1			ZO Hexane SPE fraction 2			Indomethacin		
		IC_{50} (95% CI)			IC_{50} (95% CI)			IC_{50} (95% CI)
mg/ml	% Inhibition	mg/ml	mg/ml	% Inhibition	mg/ml	mg/ml	% Inhibition	mg/ml
10.0	7.46 ± 6.45	72.14	50.0	2.66 ± 6.47	98.65	10.0	2.74 ± 2.28	73.69
25.0	22.55 ± 7.00	(66.34-78.34)	75.0	13.55 ±3.69	(90.61-107.30)	25.0	8.21 ± 2.96	(71.88-75.50)
50.0	41.52 ± 5.98		100.0	28.56 ± 7.36		50.0	27.50 ±1.30	
100.0	62.34 ±3.60		200.0	57.01 ± 6.52		75.0	48.95 ± 3.33	
200.0	71.44±7.13		300.0	68.74 ± 7.52		100.0	67.28 ±3.99	
300.0	79.82 ± 5.40		400.0	74.05 ±7.18		150.0	90.43 ± 1.94	
400.0	86.45±6.12		500.0	83.36 ± 4.38		200.0	98.42 ±1.59	
500.0	95.62 ±5.31		750.0	94.72 ±6.23				

ZO: *Zingiber officinale*, SPE: Solid-phase extraction. Values represent the mean ± SD (standard deviation) and are performed in three independent experiments; each experiment was done in triplicates (n=9) at a 95% confidence interval (CI)

Table 3A: Behavioral patterns of Swiss Webster mice treated with *Zingiber officinale* extracts and SPE fractions

Treatment group	Dose (mg/kg)	Behavioral Patterns			
		30 min	1 hr	6 hr	24 hr
ZO Methanol	2000	Hypoactivity, Respiratory changes	-	None	None
ZO Hexane	2000	Hypoactivity, Respiratory changes	-	None	None
PEG 400 (vehicle control)	-	None	-	None	None
ZO Hexane SPE fraction 1	175	-	None	None	None
	550	-	None	None	None
	2000	-	Hypoactivity, Salivation, Respiratory changes, Convulsion, Itching, Death	Hypoactivity, Respiratory changes	None
PVPPG (vehicle control)	-	-	None	None	None
ZO Hexane SPE fraction 2	2000	-	None	None	None

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

PG (vehicle control)	-	-	None	None	None
Distilled water (control)	-	-	None	None	None

Table 3B: Mortality of Swiss Webster mice treated with *Zingiber officinale* extracts and SPE fractions

Treatment group	Dose (mg/kg)	Survival (Number of animals)
ZO Methanol	2000	5/5
ZO Hexane	2000	5/5
PEG 400 (vehicle control)	-	5/5
ZO Hexane SPE fraction 1	175	1/1
	550	1/1
	2000	3/4
PVPPG (vehicle control)	-	5/5
ZO Hexane SPE fraction 2	2000	5/5
PG (vehicle control)	-	5/5
Distilled water (control)	-	5/5

ZO: *Zingiber officinale*, PEG: polyethylene glycol, PG: propylene glycol, PVPPG: polyvinylpyrrolidone in propylene glycol.

Table 4: Analgesic activity of *Zingiber officinale* extracts and SPE fractions in acetic acid-induced writhing in Swiss Webster mice

Group	Dose (mg/kg body weight)	No. of writhes in 20 min	% Inhibition
ZO Methanol	200	0.00 ± 0.00 *	100.00 ± 0.00 *
	500	0.33 ± 0.33 *	99.19 ± 0.81 *
	1000	0.00 ± 0.00 *	100.00 ± 0.00 *
ZO Hexane	200	2.17 ± 2.17 *	94.72 ± 5.28 *
	500	5.17 ± 3.10 *	87.40 ± 7.57 *
	1000	3.67 ± 2.79 *	91.06 ± 6.80 *
PEG 400 (vehicle control)		37.17 ± 3.38	9.35 ± 8.24
ZO Hexane SPE fraction 1	100	3.67 ± 1.09 *	89.37 ± 3.15 *
	250	0.17 ± 0.17 *	99.52 ± 0.48 *
	500	7.33 ± 0.49 *	78.74 ± 1.43 *
ZO Hexane SPE fraction 2	100	6.83 ± 0.70 *	80.19 ± 2.04 *
	250	2.83 ± 0.70 *	91.79 ± 2.04 *
	500	0.33 ± 0.33 *	99.03 ± 0.97 *
PEG 400 (vehicle control)		34.50 ± 3.55	0.00 ± 10.28
Diclofenac sodium	5	0.00 ± 0.00 *	100.00 ± 0.00

ZO: *Zingiber officinale*, SPE: Solid-phase extraction, PEG: polyethylene glycol. Values represent the mean ± SEM (standard error mean); n=6. * P< 0.05 as compared to the control

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

The Animal Pharmacology, Cell and Molecular Biology, and Chemistry groups of the Biological Sciences Department, Medical Affairs Division, Unilab Inc., Philippines, and LC-MS/MS facility of Pascual Pharma, Laguna, Philippines, are acknowledged for their technical support. The authors thank Dr. Cleofe Calanasan for her helpful comments on the article. This study was supported through the Grants-In-Aid (number FP 190014) Program of the Philippine Council for Health Research and Development (PCHRD), Department of Science and Technology (DOST).

References

1. Han YA, Song CW, Koh WS, Yon GH, Kim YS, Ryu SY, Kwon HJ, Lee KH. Anti-inflammatory effects of the *Zingiber officinale roscoe* constituent 12-dehydrogingerdione in lipopolysaccharide-stimulated Raw 264.7 cells. Phytother Res. 2013; 27(8):1200-1205.
2. Kizhakkayil J, Sasikumar B. Diversity, characterization and utilization of ginger: A review. Plant Genet. Resour. Characterisation Util. 2011; 9(3):64-477.
3. Johnson JB, Mani JS, White S, Brown P, Naiker M. Current Research in Food Science Pungent and volatile constituents of dried Australian ginger. Curr. Res. Food Sci. 2021; 4:612-618.
4. Mao QQ, Xu XY, Cao SY, Gan RY, Corke H, Beta T, Li HB. Bioactive compounds and bioactivities of ginger (*zingiber officinale roscoe*). Foods. 2019; 8(6):1-21.
5. Prasad S, Tyagi AK. Ginger and its constituents: Role in prevention and treatment of gastrointestinal cancer. Gastroenterol. Res. Pract. 2015; 2015:142979.
6. Yeh HY, Chuang CH, Chen HC, Wand CJ, Chena TL, Lin LY. Bioactive components analysis of two various gingers (*Zingiber officinale Roscoe*) and antioxidant effect of ginger extracts. LWT - Food Sci. Technol. 2014; 55(1):329-334.
7. Rupasinghe V, Gunathilake K. Recent perspectives on the medicinal potential of ginger. Bot. Targets Ther. 2015; 2015(5):55-63.
8. Siddiq IA, Martin O, Lawal, Usman, IN, Muhammad MB. Cardio-and-Hepatoprotective Benefits of Some Spices in Wistar Rats Induced with Metabolic Syndrome. Trop. J. Nat. Prod. Res. 2022; 6(10):1707-1714.
9. Pagano E, Souto EB, Durazzo A, Sharifi-Rad J, Lucarini M, Souto SB, Salehi B, Zam W, Montanaro V, Lucariello G, Izzo AA, Santini A, Romano B. Ginger (*Zingiber officinale Roscoe*) as a nutraceutical: Focus on the metabolic, analgesic, and anti-inflammatory effects. Phyther. Res. 2021; 35:2403-2417.
10. Fajrin FA, Nugroho AE, Nurrochmad A, Susilowati R. Ginger extract and its compound, 6-shogaol, attenuates painful diabetic neuropathy in mice via reducing TRPV1 and NMDAR2B expressions in the spinal cord. J. Ethnopharmacol. 2020; 249:112396.
11. Balberona AN, Noveno JJ, Angeles MGB, Santos RI, Cachin EJDJ, Cruz KGJ. Ethnomedicinal Plants Utilized by the Ilongot-Ẹgongot Community of Bayanihan, Maria Aurora, Aurora, Philippines. Int. J. Agric. Technol. 2018; 14(2):145-159.
12. Baleta FN, Donato JG, Bolaños JM. Awareness, utilization and diversity of medicinal plants at Palanan, Isabela, Philippines J. Med. Plants Stud. 2016; 4(4):265-269.
13. Demayo CG, Olowa L. Ethnobotanical Uses of Medicinal Plants among the Muslim Maranaos in Iligan City. Adv. Environ. Biol. 2015; 9(7):204-215.
14. Lantz RC, Chen GJ, Sarihan M, Sólyom AM, Jolad SD, Timmermann BN. The effect of extracts from ginger rhizome on inflammatory mediator production. Phytomedicine. 2007; 14(2-3):123-128.
15. Putra ED, Nazliniwaty N, Syafruddin S, Nerdy, N. (2021). Component Analysis of White Ginger (*Zingiber officinale Roscoe*) Extract and Red Ginger (*Zingiber officinale Rubra*) Extract. Trop. J. Nat. Prod. Res. 2021; 5(9):1634-1637.
16. Mehrzadi S, Khalili H, Fatemi I, Malayeri A, Siahpoosh A, Goudarzi M. Zingerone Mitigates Carrageenan-Induced Inflammation Through Antioxidant and Anti-inflammatory Activities. Inflammation. 2021; 44(1):186-193.
17. Li J, Thangaiyan R, Govindasamy K, Wei J. Anti-inflammatory and Anti-Apoptotic Effect of Zingiberene on IsoproterenolInduced Myocardial Infarction in Experimental Animals. Hum Exp Toxicol. 2020; 40(6):915-927.
18. Nishidono Y, Saifudin A, Nishizawa M, Fujita T, Nakamoto M, Tanaka K. Identification of the Chemical Constituents in Ginger (*Zingiber officinale*) Responsible for Thermogenesis. Nat. Prod. Commun. 2018; 13(7):869-873.
19. Park JS, Jung MY. Development of high-performance liquid chromatography-time-of-flight mass spectrometry for the simultaneous characterization and quantitative analysis of gingerol-related compounds in ginger products. J. Agric. Food Chem. 2012; 60(40):10015-10026.
20. Tao Y, Li W, Liang W, Van BRB. Identification and Quantification of Gingerols and Related Compounds in Ginger Dietary Supplements Using High-Performance Liquid Chromatography-Tandem Mass Spectrometry. J. Agric. Food Chem. 2009; 57(21):10014-10021.
21. de Lima RMT, Dos Reis AC, de Menezes APM, Santos JVO, Filho JWGO, Ferreira JRO, de Alencar MVOB, da Mata AMOF, Khan IN, Islam A, Uddin SJ, Ali ES, Islam MT, Tripathi S, Mishra SK, Mubarak MS, Melo-Cavalcante AAC. Protective and therapeutic potential of ginger (*Zingiber officinale*) extract and [6]-gingerol in cancer: A comprehensive review. Phytother Res. 2018; 32(10):1885-1907.
22. Yücel Ç, Karatoprak GŞ, Açıkara ÖB, Akkol EK, Barak TH, Sobarzo-Sánchez E, Aschner M, Shirooie S. Immunomodulatory and anti-inflammatory therapeutic potential of gingerols and their nanoformulations. Front Pharmacol. 2022; 13:902551.
23. Ley-Martínez JS, Ortega-Valencia JE, García-Barradas O, Jiménez-Fernández M, Uribe-Lam E, Vencedor-Meraz CI, Oliva-Ramírez J. Active Compounds in *Zingiber officinale* as Possible Redox Inhibitors of 5-Lipoxygenase Using an In Silico Approach. Int. J. Mol. Sci. 2022; 23(11):6093.
24. Ali BH, Blunden G, Tanira MO, Nemmar A. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale Roscoe*): a review of recent research. Food Chem. Toxicol. 2008; 46(2):409-420.
25. Semwal RB, Semwal DK, Combrinck S, Viljoen AM. Gingerols and shogaols: important nutraceutical principles from ginger. Phytochemistry. 2015; 117:554-568.
26. Wohlmuth H, Leach DN, Smith MK, Myers SP. Gingerol content of diploid and tetraploid clones of ginger (*Zingiber officinale Roscoe)*. J. Agric. Food Chem. 2005; 53(14):5772-5778.
27. Bischoff-Kont I, Fürst R. Benefits of Ginger and Its Constituent 6-Shogaol in Inhibiting Inflammatory Processes. Pharmaceuticals. 2021; 14(6):571.
28. Flynn DL, Rafferty MF, Boctor AM. Inhibition of human neutrophil 5-lipoxygenase activity by gingerdione, shogaol, capsaicin and related pungent compounds. Prostaglandins, Leukot. Med. 1986; 24(2-3):195-198.
29. Weidner MS, Sigwart K. Investigation of the teratogenic potential of a *Zingiber officinale* extract in the rat. Reprod. Toxicol. 2001; 15(1):1575-1580.
30. Wilkinson JM. Effect of ginger tea on the fetal development of Sprague–Dawley rats. Reprod. Toxicol. 2000; 14(6):507-512.
31. Chrubasik S, Pittler MH. Roufogal BD. *Zingiberis rhizoma*: a comprehensive review on the ginger effect and efficacy profiles. Phytomedicine. 2005; 12(9):684-701.
32. Zick SM, Djuric Z, Ruffin MT, Litzinger AJ, Normolle DP, Alrawi S, Feng MR, Brenner DE. Pharmacokinetics of 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol and conjugate metabolites in healthy human subjects. Cancer

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Epidemiol. Biomark. Prev. 2008; 17(8):1930-1936.
33. Sánchez-Mateo CC, Bonkanka CX, Hernández-Pérez M, Rabanal RM. Evaluation of the analgesic and topical anti-inflammatory effects of *Hypericum reflexum L*. fil. J. Ethnopharmacol. 2006; 107(1):116.
34. Young HY, Luo YL, Cheng HY, Hsieh WC, J Liao JC, Peng WH. Analgesic and anti-inflammatory activities of [6]-gingerol. J. Ethnopharmacol. 2005; 96(1-2):207-210.
35. Suekawa M, Ishige A, Yuasa K, Sudo K, Aburada M, Hosoya E. Pharmacological studies on ginger. I. Pharmacological actions of pungent constitutents, (6)-gingerol and (6)-shogaol. J Pharmacobiodyn. 1984;7(11):836-848.

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License