Journal of Animal & Plant Sciences, 34(6): 2024, Page: 1450-1460
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2024.6.0823

INVESTIGATING *ZINGIBER OFFICINALE* RHIZOME'S PHYTOCHEMICAL MAKE-UP AND BIOACTIVITY: A COMPARISON OF AQUEOUS AND ETHANOL EXTRACTS FOR ANTIFUNGAL, ANTI-INFLAMMATORY, AND ANTIBACTERIAL PROPERTIES

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ABSTRACT

Zingiber officinale or ginger is used to treat many ailments, including cancer, heartburn, inflammation, nausea, and vomiting, as it is enriched in polyphenols, terpenes, flavonoids and essential oils. The study uses ethanol and water extracts of *Zingiber officinale* rhizome to analyze the phytochemical composition and antibacterial, anti-dandruff, and anti-inflammatory properties. The extracts were prepared by maceration. Using reference techniques, proximate analysis and phytochemical evaluation were conducted. *Malassezia furfur* was used for anti-dandruff testing, while antibacterial sensitivity was assessed against *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. For anti-inflammatory activity, the protein denaturation method was employed. The extract with the highest activity underwent GC/Mass analysis to understand its phytochemical profiling. The study demonstrated the presence of valuable phytochemicals such as polyphenols, glycosides, flavonoids, and tannins. The ethanol extract exhibited larger inhibition zones (mm) than the water extract at all concentrations, the most significant differences ($p < 0.05$) occurring at 200 mg/mL: antifungal activity was 19.4±0.77 vs 12.8±0.65, and antibacterial activity was 19.4±0.49 vs 17.2±0.58 for *Pseudomonas aeruginosa* and 20.1±0.75 vs 18.3±0.89 for *Streptococcus pneumoniae*. The ethanol extract (86.8%±0.038) and the water extract (80.2%±0.029) substantially reduced inflammation ($p < 0.05$). The findings of these assays were comparable to relative standard drugs, including fluconazole (20.7±0.81 mm), ciprofloxacin (21.5±0.67 mm), and diclofenac (90.1%±0.003). The GC/Mass analysis of the ethanol extract revealed significant amounts of gingerol, gingerdione, sitosterol, and zingerone. This study highlights *Zingiber officinale's* potential therapeutic effects, advocating its use as a beneficial alternative to synthetic drugs.

Keywords: *Zingiber officinale*, Traditional medicine, Antibacterial, Antifungal, Anti-inflammatory.

Published first online October 22, 2024 Published final December 22, 2024

INTRODUCTION

Medicinal plants have been the primary source of healing substances for millions of years. They have various bioactive molecules with different pharmacological and therapeutic capabilities. The World Health Organization (WHO) has acknowledged the benefits of traditional medicine, making healthcare affordable to patients everywhere. WHO emphasizes the need for evidence-based procedures, safety, and regulation to certify the efficient and responsible use of traditional medicine while advocating for its inclusion in national healthcare systems (Ashiq *et al.,* 2022; Nabi *et al.,* 2023). Because the majority of people cannot afford prescription medicines, they continue to use traditional plant-based cures. Beyond their use in medicine, medicinal plants support sustainable lifestyles, biodiversity conservation, and economic growth, especially in areas where they are essential resources for local communities and traditional healers. These natural resources provide essential treatments for various illnesses, from minor aches and pains to severe diseases. Additionally, medicinal plants are crucial in conventional medical systems because they support endemic healthcare practices and the preservation of cultural legacy (Ashiq *et al.,* 2021; Qurat-ul-Ain *et al.,* 2022).

A perennial herbaceous plant in the Zingiberaceae family, ginger (*Zingiber officinale*) is well-known for its culinary and medicinal properties. Tropical and subtropical climates are ideal for ginger growth, and it prefers rich soils with good drainage and shade. It is frequently grown in regions like Southeast Asia, India, China, and specific areas of Africa and the Caribbean that are renowned for having warm temperatures and high humidity. From its rhizomatous underground stem, the plant has fragrant cone-shaped blossoms with purple scoring on their yellow or white petals. However, the rhizome primarily displays the majority relevance as an

altered underground stem. This rhizome, which goes by the colloquial name "ginger root", is distinguished by its irregular texture, pale yellow flesh, and pungent smell. Its long, leafy stems, which can reach a height of one meter, are adorned with alternate lanceolate leaves with distinct midribs and sheathing bases (Sommano and Tangpao, 2021).

Zingiber officinale is renowned for having a complex phytochemical composition that includes a variety of secondary metabolites with a wide variety of biological and chemical characteristics. The primary bioactive ingredients in ginger, known as gingerols, are phenolic chemicals that give ginger its unique aroma and powerful medical properties. Gingerols are important subjects of pharmacological research because of their potent anti-inflammatory, antioxidant, and anticancer characteristics (Deng *et al.,* 2022).

Ginger is a highly valued medicinal herb firmly ingrained in the cultural legacies of many different nations, as seen by the abundance of folklore surrounding it. Ginger has long been used in traditional medical practices worldwide because of its wide range of health benefits. Ginger's capacity to enhance digestion, lessen nausea and treat respiratory conditions is noteworthy in the context of the ancient Indian medical system known as Ayurveda. Ginger is regarded in Traditional Chinese Medicine (TCM) as a warming plant that promotes circulation, relieves pain, and cures colds. Throughout history, ginger has been used to treat gastrointestinal pain, including indigestion, bloating, and motion sickness. In addition, it has been used as a natural remedy for inflammatory illnesses like arthritis and as a warming agent in the fight against colds and the flu (Shahrajabian *et al*., 2019; Yasmeen *et al*., 2020).

Zingiber officinale has a diverse range of pharmacological effects due to the presence of various bioactive components like shogaols, paradols, zingerone, volatile oils, and gingerols. When gingerols are dehydrated during processing or storage, they yield shogaols, which have more bioactivity than the precursor chemicals. Another class of phenolic substances in ginger, called paradols, has anti-inflammatory and anticancer properties via various pathways. Ginger's volatile oils, such as zingiberene, β-sesquiphellandrene, and α-curcumene, contribute to its distinct smell and possess antibacterial qualities (Zhang *et al.,* 2021). The combined effects of these volatile ingredients and non-volatile phenolic compounds increase the overall therapeutic potential of ginger extracts. Its potent anti-inflammatory properties are among its most remarkable qualities, mainly due to gingerols and other related chemicals. Ginger may be a viable treatment for inflammatory diseases like arthritis and inflammatory bowel disease because these components inhibit inflammatory pathways, including producing pro-inflammatory cytokines and enzymes (Ballester *et al.,* 2022). Ginger also exhibits potent antiemetic properties. Its ability to improve digestion and lessen gastrointestinal pain supports its historical use as a digestive remedy (Nocerino *et al.,* 2021). Additionally, ginger demonstrates antibacterial qualities that prevent the growth of many bacteria and fungi, potentially supporting its anti-infective medicinal efficacy. Overall, ginger's pharmacological properties indicate that it has the potential to be an effective natural remedy for a variety of illnesses (Khan *et al.,* 2021; Ballester *et al.,* 2022).

Even though *Zingiber officinale*'s pharmacological qualities have been studied in great detail, thorough comparison studies evaluating the bioactivity of ethanol and aqueous extracts are still required. When creating standardized herbal formulations and maximizing the medicinal potential of ginger extracts, it's critical to comprehend the differences in phytochemical content and bioactivity between these two solvent systems. Additionally, elucidating the processes behind ginger extracts' anti-inflammatory, antifungal, and antibacterial properties can provide an essential context for understanding their therapeutic uses and aid in creating novel phytopharmaceutical formulations. Hence, this study's primary goal is to conduct a phytochemical analysis of rhizomes' of *Zingiber officinale* using aqueous and ethanol extracts and evaluate the both extracts' antibacterial, antifungal, and anti-inflammatory properties.

MATERIALS AND METHODS

Instruments: Electric balance (Eppendorf, Germany), UV visible spectrophotometer (UV-2550, Agilent Technologies, Germany), sonicator, incubator (MIR-153 Sanyo Electric Corporation, Japan), and autoclave (Eppendorf, Germany) were used during this study.

Chemicals: Ethanol (Sigma-Aldrich), Chloroform (BDH), Methanol (Merck), Sulphuric acid (BDH), Ciprofloxacin disk (Merck), Fluconazole disk (Sigma-Aldrich). Muller Hinton agar (Sigma-Aldrich) and Sabouraud dextrose agar (Sigma-Aldrich).

Microorganisms strains: Microbiological strains were obtained from the Chaudhry Muhammad Akram Teaching and Research Hospital, which is affiliated with Superior University in Lahore, Pakistan. *Malassezia furfur* for dandruff (ATCC 14521), *Pseudomonas aeruginosa* (ATCC 10145) and *Streptococcus pneumoniae* (ATCC 49619) are the strains used in this investigation for antibacterial activity.

Standardization of inoculum and media preparation: The instructions and specifications provided by the manufacturer were followed for preparing and using the microbial medium. Following the guidelines of the Clinical and Laboratory Standard Institute (CLSI, 2015),

the microbiological turbidity of each species was formed and standardized (Humphries *et al.,* 2018).

Plant collection and authentication: Dr. Zaheer-ud-Din Khan, a botanist at GC University Lahore in Punjab, Pakistan, authenticated *Zingiber officinale* and assigned it voucher number 3921. The collection involved 1.5 kilograms of fresh rhizome during March 2022, which was subsequently dried in the shade for 21 days. The dried material was then ground into a powder using a grinder and stored in an airtight jar for future use.

Extraction: The extracts were made via the maceration technique. Two equal amounts of 1kg of rhizome powder were separated and placed inside different glass flasks. The plant material is then thoroughly soaked in the solvent by dissolving the dry powder in ethanol and water for seven days at room temperature. Each flask was stirred from time to time. After that, each extract was strained through filter paper to eliminate any particles. A rotary evaporator was used to concentrate the extracts, which were then stored at 4°C in vials labelled appropriately (Tanweer *et al*., 2020).

Percentage yield of extracts: The percentage yield of aqueous and ethanolic extracts was calculated by using the following formula:

Percentage yield = (Theoretical yield/Actual yield) ×100

Proximate analysis: USP (2015) was followed for the proximate analysis of the rhizome powder (USP, 2015). The complete detail is given below:

Total ash contents: 2g of rhizome powder was added to a tarred silica crucible. Until all of the carbon was removed, the material was burned in a muffle furnace at 625±25°C. Before being weighed, the crucible was allowed to cool to room temperature. To estimate the contents, the following formula was applied.

Total **ash (%) = (weight of ash ÷ weight of sample) x 100**

Acid insoluble ash: All ash obtained from the previous technique was left in 25 mL of diluted hydrochloric acid for five minutes. The ashless filter paper was used to obtain a residue. After that, the residue was cleaned up using hot, distilled water. The material was then transferred into a silica crucible, dried in an oven, and burned in a muffle furnace at 625±25°C to eliminate any remaining carbon. The crucible temperature was brought down to 25°C, and the following formula was used to estimate the final temperature.

Acid insoluble ash (%) = (weight of ash ÷ weight of sample) x 100

Water insoluble ash: For 5 minutes, the entire amount of insoluble ash was heated in 25 mL of distilled water. The ashless filter paper was utilized to obtain extract residue. Before the contents were added to the silica crucible, any leftovers were cleaned out with hot distilled water. Next, in order to eliminate any leftover carbon, the crucible was put in the muffle furnace set at 625±25°C. Once taken out, it was allowed to cool in a desiccator. The final assessment was conducted using the following formula.

Water insoluble ash (%) = (weight of ash ÷ weight of the sample) x 100

Sulphated ash: After carefully weighing 2g of the rhizome powder, it was added to the silica crucible that had been tarred. The following step involved repeating this procedure twice: the contents were immersed in concentrated sulfuric acid and burnt gently until the white vapours ceased dissipating. At 625±25ºC, the crucible was kept in a muffle furnace until the contents were carbon-free. After that, the crucible was taken out and allowed to cool. The last computation was performed using the following equation.

Sulphated ash (%) = (weight of sulphated ash/weight of the sample) x 100

Moisture content analysis: After carefully weighing the 2g of plant rhizome powder, put it in the tarred china dish. After that, place the china dish at 105°C in the oven. After thirty minutes, the porcelain dish was removed and allowed to come to room temperature in a desiccator. Once the contents have cooled, weigh the china dish once again. The following formula was used to determine the percentage of moisture content.

Dry matter (%) = weight of air-dried sample - weight of air-dried sample) x 100

Qualitative tests for the estimation of metabolites: The standard procedures were adopted for phytochemical screening (Shaikh and Patil, 2020).

Test for saponins: A test tube was filled with 5 mL of water and approximately 0.5g of powdered rhizome. After this, add three drops of olive oil and shake well until produced frothing. Finally, the development of an emulsion was noticed.

Test for tannins: 0.5g of rhizome powder was boiled in 10 mL of H_2O in a test tube. The solution was then filtered. Ferric chloride (0.1%) drops (a few) were then added. Finally, the solution's blue-black or brownish-green hue was examined.

Test for anthraquinones: 0.5g of the rhizome powder was boiled with 10 mL of H_2SO_4. The hot solution was then filtered and shaken with chloroform (5 mL). After this, the chloroform layer was pipetted in another test tube. Finally, dilute ammonia (1 mL) was added and then watched for color changes.

Test for steroids: In 10 mL of chloroform, about 0.5g of the rhizome powder was dissolved, and then, from the sides of the test tubes, equal volumes of concentrated sulfuric acid was added. A yellowish sulphuric acid layer with green fluorescence and upper reddish color indicated the presence of steroids in the sample. This test was used to identify cardiac glycosides.

Keller-Killiani Test: The first step was to dissolve rhizome powder (0.5g) in water (5 mL). The next step involved adding a glacial acetic acid solution (2 mL) containing one drop of ferric chloride solution. Then, this solution was under-layered with concentrated sulfuric acid (1 mL). Ultimately, the presence of a brown ring at the interface was noted in this solution, indicating the presence of deoxysugar, a feature common to cardenolides.

Anti-dandruff activity: The disk diffusion method was employed to quantify the extracts' anti-dandruff efficacy. After drying for seven hours, sterile paper discs (6 mm in diameter) impregnated with 100 µl of each diluted extract at concentrations of 50 mg/mL, 100 mg/mL and 200 mg/mL were placed on the inoculated agar plate. The standardized inoculum was applied to the surface of agar plates using a sterile swab, and the test isolate was then added to SDA plates that had been coated with 1 mL of olive oil. As 32°C is the optimal temperature for *Malassezia furfur* development, the 48-hour incubation was conducted at this temperature. This assay used a disk containing sterile water as the negative control, whereas a disk containing fluconazole (40 mg/mL) was used as the positive control. The antifungal activity was determined using zone of inhibition measurements, and the test was repeated three times to find the mean and standard deviation (Dahal *et al.,* 2021).

Antibacterial assay: After isolating each bacterium into a pure colony, it was maintained in sterile saline until the turbidity reached McFarland tube number 0.5, equivalent to 1.5 x 108 CFU/mL. A loopful of each adjusted organism was swabbed onto Muller Hinton agar. The inoculated agar plate was covered with sterile paper disks (6 mm) impregnated with 100 µl of diluted extract at 50 mg/mL, 100 mg/mL, and 200 mg/mL. The disks were then dried in a hot air oven at 100°C for two hours. Following that, the plates were incubated per each organism's growth requirements. Considering the 6 mm disk, the zones of inhibition for each sample were measured and recorded in millimetres (mm) to determine its antibacterial activity. Three separate tests were run for each test. A parallel analysis used the commercially available antibacterial drug ciprofloxacin (5µg disk) as a positive control. As a negative control, a sterile distilled water disk was employed. The inhibition zones were calculated as means and standard deviation (Hemeg *et al.,* 2020).

Anti-inflammatory activity: The extract (1%), 2.8 mL of phosphate-buffered saline, and 0.2 mL of fresh egg albumin were all included in the reaction mixture (5 mL). The same amount of twice-purified water was utilized as a control. After being incubated for 15 minutes at 37±2°C, the mixture was heated for 5 minutes at 70°C. Then, all solutions were cooled, and their absorbance was measured using a 660 nm wavelength against a vehicle blank. In this experiment, diclofenac sodium was utilized as the reference drug. The following formula was used to determine the percentage of protein denaturation inhibition. (Latif *et al.,* 2020).

% inhibition = [(Absorbance $_{(control)}$ - Absorbance $_{(test)}$ / Absorbance $_{(control)}$] x 100

Gas chromatography-mass spectroscopy (GC/Mass) analysis: A GC/Mass analysis was performed on the most active extract to assess the phytochemical profiling. A 30 m × 250 µm capillary column covered with a 0.25-µm layer of 5% phenyl methylpolysiloxane was used to separate the analytes. Split injection was applied to the samples at a 30:1 ratio. The column oven temperature was set at a pace of 15 minutes per degree Celsius. It began at 50°C and was maintained for two minutes before rising to 300°C, which was held there for five minutes. The temperature of the ion source and injection was set to 250°C. The carrier gas was 99.99% helium flowing at a rate of 1 mL min−1. In full-scan acquisition mode, the mass range scanned was 40–400 amu, with the ionization electron energy set at 70 eV. The National Institute of Standards and Technology (NIST, Washington, DC) mass spectrum library was used to identify the compounds (van Den Dool and Kratz, 1963).

Statistical analysis: The statistical analysis was performed using Graph Pad Prism version 8 and Microsoft Excel 2016. All the experiments were conducted in triplicates (n=3), and results were presented as mean±standrad deviation. Using one-way ANOVA, statistical differences were calculated, followed by Tukey's post hoc analysis. A value of p less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Extraction yield: The rhizomes of Zingiber officinale were extracted by maceration using ethanol and water as solvents. The percentage yield results are shown in Figure 1. Solvent selectivity in the extraction process influences the phytochemical content of the extracts in a significant way. In the present investigation, aqueous and ethanol solvents were used to extract bioactive compounds from the rhizomes of Zingiber officinale. Water is selective in dissolving polar compounds; on the other hand, ethanol, due to its less polarity, comes as a better solvent where it can dissolve various polar and

non-polar compounds, which makes the possibility of the ethanol extract being enriched with diverse bioactive compounds (Ravishanker *et al*., 2013; Arif *et al*., 2024).

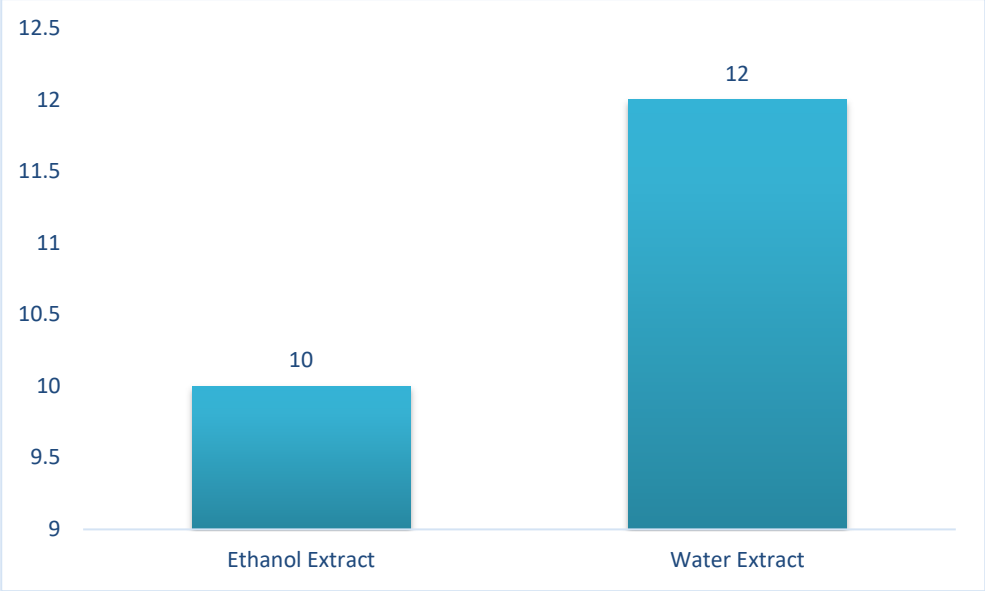

Figure 1. Percentage yield of the extracts

Physicochemical testing: Proximate analysis was done to standardize the powder made from dried leaves. Values for total ash, acid-insoluble ash, and water-soluble ash are tabulated in Table 1.

Table 1. The results of the physicochemical analysis of rhizome powder (n=3)

Physicochemical Variables	Contents (weight/weight %)
Ash total value	3.08 ± 0.078
Acid insoluble ash value	3.4± 0.061
Ash water-soluble value	5.43 ± 0.089
LOD (loss on drying value)	8.23 ± 0.065
Extractive value (water soluble)	23.2 ± 0.084
Extractive value (alcohol value)	19.87 ± 0.079

Various ash and extractive values were calculated to determine the quality, purity, and polarity of the rhizome powder. The assessment of ash values holds significant importance in detecting the presence of foreign materials such as silica or oxalate. The total ash value of the tested plant was determined to be 3.08%, indicating that it falls within the official range (less than 20%) as recommended according to the USP (Nikam, Kareparamban *et al.* 2012). High values for alcohol and water solubility suggest the presence of polar compounds such as glycosides, tannins, and phenols (Chanda and Baravalia, 2011; Latif *et al.,* 2020).

Phytochemical analysis: Table 2 displays the findings of a phytochemical analysis of the extracts. The findings showed that the extracts contained phytochemicals, including phenolics, proteins, carbohydrates, tannins, flavonoids, and saponins, which may be helpful in treating various ailments.

Table 2. The presence of the phytochemicals in ethanolic and aqueous extracts.

Phytochemicals	Ethanol extract	Aqueous extract
Alkaloids	++	-
Carbohydrates	+	+++
Flavonoids	+++	++
Glycoside	+	-
Phenols	++	+++
Polyphenols	+++	+++
Proteins	+	++
Saponins	++	+
Tannins	++	++
Terpenoids	++	+

Historically, before their significance in science was recognized, secondary metabolites were considered surplus products of plants. Subsequently, their importance was acknowledged, highlighting their crucial role in various biological processes (Mahajan *et al.,* 2020). The phytochemical analysis encompassed the estimation of both primary and secondary metabolites. The evaluation of primary metabolites included the

determination of proteins, lipids, and carbohydrates. Concurrently, the assessment of secondary metabolites comprised polyphenols, polysaccharides, flavonoids, and glycosaponins, with the findings summarized in Table 2. The qualitative examination of primary metabolites unveiled that the rhizome powder of the plant contained carbohydrates and proteins. The high nutritional value of the plant is indicated by the presence of these primary metabolites (Odhav *et al.,* 2007; Salam *et al.,* 2023). Polyphenols and flavonoids were found in extracts from *Zingiber officinale* rhizomes, with the aqueous extract producing the same intensity of reaction as the ethanolic extract, according to analyses of secondary metabolites. The fact that flavonoids were consistently present in both solvents showed that ethanol and water were equally successful in removing these beneficial substances from the plant material (Mufson, 1981). A wide variety of secondary metabolites have been produced by medicinal plants, exhibiting a wide range of biological activity and therapeutic effects (Wink, 2015). Among these secondary metabolites, polyphenols stand out as a diverse and abundant group. They exhibit notable anti-inflammatory properties, providing protection against conditions such as arthritis (Bravo, 1998; Bucciantini *et al.,* 2021). Additionally, polyphenols have demonstrated anti-tumor effects, reduction of gout symptoms (Oliviero *et al.,* 2018), and cardiovascular protective effects (Manach *et al.,* 2005). The presence of significant phenolic contents in the plant indicates a natural source of potential antioxidants (Boğa *et al.,* 2011; Bucciantini *et al.,* 2021.

Alkaloids were significantly lacking in the water extract but showed a high abundance in the ethanol extract. The ethanol extract included glycosides, whereas the aqueous extract contained none. While saponins were detected in the water extract, they showed intense reactions in the ethanol extract. Both the ethanol and the water extracts had high concentrations of tannins. Regardless of the solvent used, large amounts of tannins, which are known for their astringent qualities, were discovered in both extracts, indicating a rich presence of these polyphenolic chemicals in the plant material (Surachman and Dewi, 2022). The presence of glycosaponins in the plant indicated that the therapeutic herb also has hemolytic properties (Okwu and Morah, 2004; Latif *et al.,* 2020). Additionally, research has shown that saponins have several advantages, such as antimicrobial, anti-tussive, immunomodulatory, and anti-diabetic benefits (Upadhyay *et al.,* 2018).

Antifungal activity: Table 3 outlines the anti-dandruff activity of ethanolic and water extracts across varying concentrations, measured in mg/mL. The ethanolic extract revealed a modest zone of inhibition at the minimum concentration of 50 mg/mL (7.6±0.67 mm), indicative of some antifungal impact. Notably, as concentrations increase to 100 mg/mL and 200 mg/mL, a substantial enhancement in anti-dandruff efficacy was observed ($p < 0.05$), with larger zones of inhibition measuring 13.5±0.87mm and 19.4±0.77 mm, respectively. Conversely, the water extract generally displayed smaller inhibition zones (5.6±0.55 mm at 50 mg/mL, 8.8±0.58 mm at 100 mg/mL, and 12.8±0.65 at 200 mg/mL) than the ethanolic extract at equivalent concentrations. Fluconazole consistently exhibited a high antifungal activity with a zone of inhibition of 20.7±0.81 mm. While the ethanolic extract tends to show higher antifungal efficacy, the collective results emphasize the potential of both extracts as anti-dandruff agents as both extracts are enriched with valuable phytochemicals like polyphenols and flavonoids (Jantan *et al.,* 2003; Redondo-Blanco *et al.,* 2020).

Table 3. The results of the antifungal activity (n=3)

Concentration of the extract (mg/ml)	Ethanolic Extract Zone of inhibition (mm)	Water Extract Zone of inhibition (mm)
50	7.6 ± 0.67^{a}	5.6 ± 0.55^{a}
100	13.5 ± 0.87^{ab}	8.8 ± 0.58^{ab}
200	$19.4 \pm 0.77^{ns/b}$	12.8 ± 0.65^{ab}
Fluconazole (40 mg/mL)	20.7 ± 0.81	20.7 ± 0.79

The results of the standard drug, fluconazole, were compared to the results of both extract-treated groups, and the superscript letter "a" indicated that there were statistically significant differences ($p < 0.05$). The superscript letter "ns" denoted the lack of significance of the outcomes as compared to the fluconazole. The substantial statistical difference ($p < 0.05$) between the results of two extracts in the same row is donated by the superscript letter 'b'.

Antibacterial activity: The results of the antibacterial activity are presented in Table 4. The provided data elucidates the zone of inhibition of ethanol and water extracts concerning *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*. Both extracts demonstrated substantial antibacterial activity ($p < 0.05$). For the ethanol extract, the zone of inhibition against *Pseudomonas aeruginosa* showed a concentration-dependent response and highest inhibition (19.4±0.49 mm) observed at 200 mg/mL. The aqueous extract also showed a concentration-dependent response, and the highest inhibition (17.2±0.58 mm) was observed at 200

mg/mL concentration. However, the overall effect of aqueous extract was less than that of ethanolic extract.

Similarly, the maximum zone of inhibition against *Streptococcus pneumoniae* was showed by ethanolic extract (20.1±0.75 mm) at 200 mg/mL concentration compared to aqueous extract 18.3±0.89 mm at the same concentration ($p < 0.05$). The results of the current investigation were comparable to the standard drug, ciprofloxacin. These results highlight the extraction solvents' critical role in determining how effective the extracts are as antimicrobial agents, with the ethanolic extract showing the highest efficacy (Redondo-Blanco *et al.,* 2020). The antibacterial action may be attributed to several phytochemicals, i.e. polyphenols, flavonoids and saponins. More research is necessary to determine the precise bioactive components and evaluate safety concerns. This will help to clarify the possible applications of these extracts as antimicrobial agents (Alkandahri *et al.,* 2020; Naz *et al.,* 2023).

Anti-inflammatory activity: Table 5 displays the outcomes of the anti-inflammatory activity. Both ethanolic (86.8% ± 0.038) and aqueous extracts (80.2% ± 0.029) had significant activity against inflammation ($p < 0.05$), and the results were comparable to the reference drug (90.1% ± 0.003). Plant-based phytochemicals, which are naturally occurring molecules, are essential for lowering inflammation in the body. Among these, flavonoids and polyphenols stand out for having potent anti-inflammatory properties. Phytochemicals such as flavonoids and polyphenols, found in abundance in plants like ginger, are essential in reducing inflammation within the body. Anti-inflammatory effects are also produced by the bioactive component of ginger called gingerol, which suppresses inflammatory pathways (Abdalla and Abdallah, 2018).

Table 4. The outcomes of the antimicrobial activity (n=3)

Concentration of the extract (mg/ml)	Ethanolic Extract Zone of inhibition (mm)	Water Extract Zone of inhibition (mm)
	Antibacterial activity against *Pseudomonas aeruginosa*	
50	6.1 ± 0.26^{ab}	3.5 ± 0.76^{ab}
100	11.8 ± 0.55^{ab}	9.2 ± 0.65^{ab}
200	$19.4 \pm 0.49^{ns/b}$	$17.2 \pm 0.58^{ns/b}$
Ciprofloxacin 5µg disc	21.5 ± 0.67	21.8 ± 0.63
	Antibacterial activity against *Streptococcus pneumonia*	
50	7.3 ± 0.87^{ab}	4.9 ± 0.59^{ab}
100	13.6 ± 0.76^{ab}	8.2 ± 0.64^{ab}
200	$20.1 \pm 0.75^{ns/b}$	$18.3 \pm 0.89^{ns/b}$
Ciprofloxacin 5µg disc	21.2 ± 0.62	20.5 ± 0.67

The results of the standard drug, ciprofloxacin, were compared to the results of both extract-treated groups, and the superscript letter "a" indicated that there were statistically significant differences ($p < 0.05$). The superscript letter "ns" denoted the lack of significance of the outcomes as compared to the ciprofloxacin. The substantial statistical difference ($p < 0.05$) between the results of two extracts in the same row is donated by the superscript letter 'b'.

Table 5. The results of the anti-inflammatory activity (n=3)

Extract/Standard	Mean % inhibition of protein denaturation±SD
Control	--
Ethanol	86.8 ± 0.038^{a}
Aqueous	80.2 ± 0.029^{a}
Diclofanac	90.1 ± 0.003^{a}

In comparison to the positive control group, the results of diclofenac and both extract-treated groups showed statistically significant differences ($p < 0.05$), as indicated by the superscript letter "a".

GC/Mass analysis: The ethanolic extract underwent GC/mass analysis because our preliminary tests showed that it exhibited significant pharmacological activities and was enriched with essential phytochemicals. The results of the GCMS analysis of the ethanolic extract of the plant are shown in Figure 2, and the major compounds are summarized in Table 6. The study findings have indicated that the rhizome contained more than eighty compounds, including gerinol, 2-pentene, beta farnesene, cyclohexane-methanol, 2-butanone, gamma.-terpinene, and n-hexadecanoic acid, etc. These compounds are known to have anti-inflammatory, antifungal, antibacterial, anti-diabetic, antilipolytic, analgesic, and antispasmodic effects (Kabuto *et al.,* 2005).

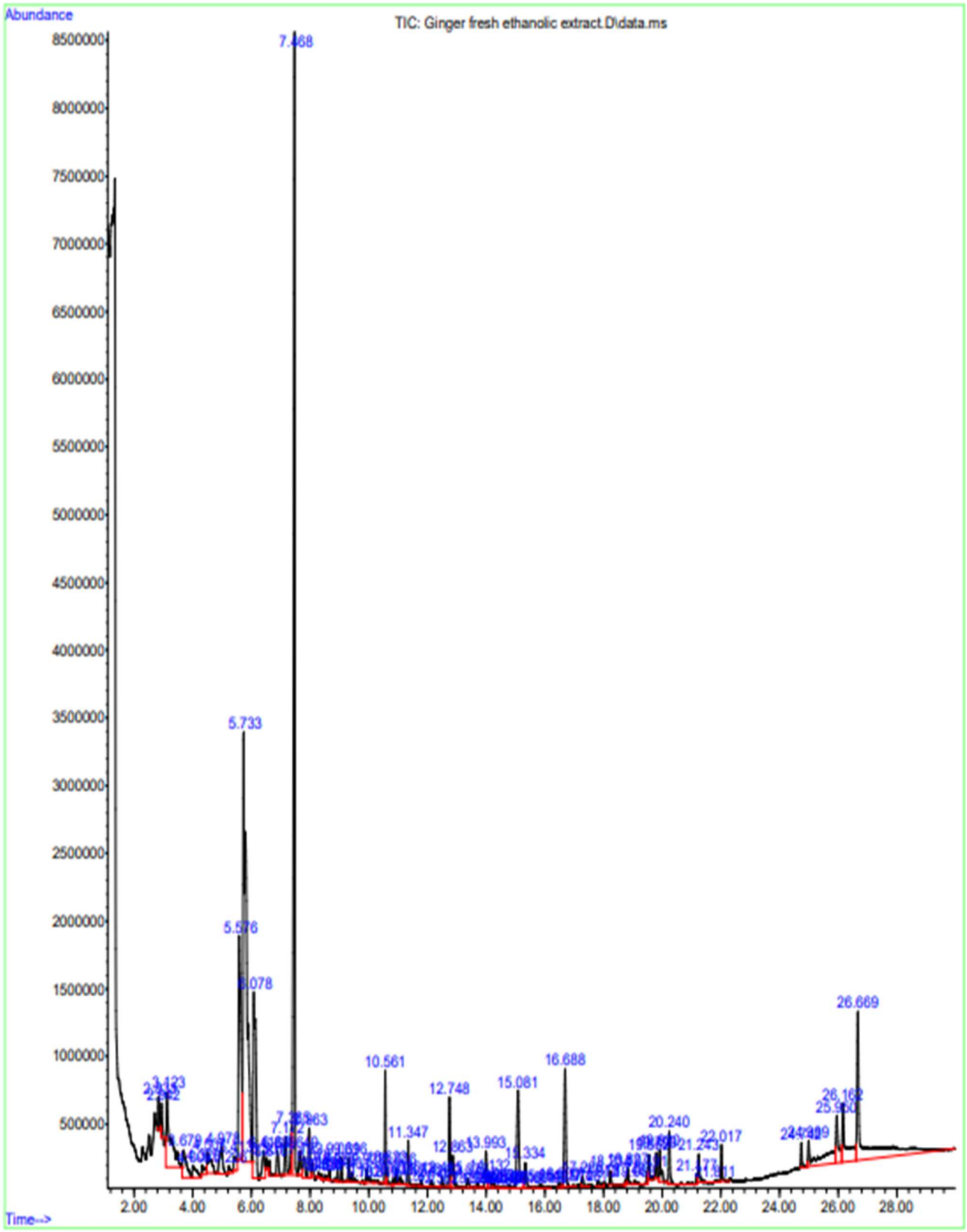

Figure 2. GC/Mass analysis of the ethanol extract of *Zingiber officinale* rhizomes

Table 6. Significantly present compounds identified by GC/Mass analysis of the ethanol extract of *Zingiber officinale* rhizomes.

Peak No.	RT (min)	Name of the compound	Molecular formula	Molecular weight (g/mol)	Area (%)
3	3.123	Citral	$C_{10}H_{16}O$	152.24	3.68
11	5.576	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl	$C_{15}H_{22}$	202.3352	6.75
12	5.733	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	$C_{15}H_{24}$	204.3511	17.94
13	6.078	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	$C_{15}H_{24}$	204.3511	5.80
20	7.468	2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)-	$C_{11}H_{14}O_3$	194.2271	18.88
36	10.561	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507	1.23
41	11.347	Ethyl tridecanoate	$C_{15}H_{30}O_2$	242.3975	0.62
47	12.748	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.4721	1.47
48	12.863	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.4879	0.90
53	13.993	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308.4986	0.74
61	15.081	[2]-Gingerdione			2.86
62	15.334	3-Decanone, 1-(4-hydroxy-3-methoxy phenyl)	$C_{17}H_{26}O_3$	278.3865	0.65
67	16.688	1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one	$C_{17}H_{24}O_3$	276.3707	2.50
79	19.920	(3R,5S)-1-(4-Hydroxy-3-methoxyphenl)decane-3,5-diyl diacetate	$C_{21}H_{32}O_6$	380.4752	0.65
86	24.989	(E)-4-(2-(2-(2,6-Dimethylhepta-1,5-dien-1-yl)-6-pentyl-1,3-dioxan-4-yl)ethyl)-2-methoxyphenol	$C_{27}H_{42}O_4$	430.6200	2.67
87	25.950	Campesterol	$C_{28}H_{48}O$	400.6801	1.41
88	26.162	Stigmasterol	$C_{29}H_{48}O$	412.6908	2.72
89	26.669	gamma. –Sitosterol			8.45

Conclusion: Both the culinary sector and conventional medicine make extensive use of ginger. It has several therapeutic advantages. The present investigation demonstrates noteworthy anti-inflammatory, antibacterial, and antifungal properties in both aqueous and ethanolic extracts. The identification of various phytochemicals underscores the plant's pharmacological actions. Further studies embracing the pharmacophore characterization and ADMET profiling may reveal additional benefits of the plant extracts in treating various ailments and their pharmacokinetic prospects. Additionally, more comprehensive research studies are required to validate the plant's safety, efficacy, and potential use in clinical practice.

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