



EXPLORING THE THERAPEUTIC POTENTIAL OF GINGER (*ZINGIBER OFFICINALE*) OIL AGAINST INFLAMMATORY PROCESS

Hamza Afzaal¹, Momina Shahid^{2*}, Nouman Mushtaq³, Saira Arshad⁴, Saleha Hameed⁵,
Ayesha Aslam⁶, QuratulAin Shahid⁷, Fatima Abid⁸

¹Hamza Afzaal (hamzaafzaal10@gmail.com) Department of Nutritional Sciences, Faculty of Life Sciences, Government College University Faisalabad, Pakistan

^{2*}Momina Shahid (mominamalik14@yahoo.com) University Institute of Diet & Nutritional Sciences, The University of Lahore, Pakistan

³Nouman Mushtaq (noumanmushtaqfsd@gmail.com) University Institute of Diet & Nutritional Sciences, The University of Lahore, Pakistan

⁴Saira Arshad (sairaammar60@gmail.com) University Institute of Diet & Nutritional Sciences, The University of Lahore, Pakistan

⁵Saleha Hameed (hameed.saleha@yahoo.com) School of Human Nutrition & Dietetics, Minhaj University Lahore, Pakistan

⁶Ayesha Aslam (Ayesha.aslam@dnsc.uol.edu.pk) University Institute of Diet & Nutritional Sciences, The University of Lahore, Pakistan

⁷QuratulAin Shahid (Quratulain.shahid@dnsc.uol.edu.pk) University Institute of Diet & Nutritional Sciences, The University of Lahore, Pakistan

⁸Fatima Abid (Fatima.abid@dnsc.uol.edu.pk) University Institute of Diet & Nutritional Sciences, The University of Lahore, Pakistan

***Corresponding Author: Momina Shahid**

***Email: mominamalik14@yahoo.com**

ABSTRACT

This research shows the effect of ginger (*zingiber officinale*) on inflammation by using various methods, like laboratory experiments and data analysis techniques. Then through HPLC (high-pressure liquid chromatography), the quantity is determined and by in-vitro tests such as the DPPH test, peroxide value test and FRAP are performed to assess gingerol's and other phenolic compound's anti-inflammatory and anti-oxidant properties. The study findings reveal that ginger oil, rich in bioactive compounds can act against inflammatory responses by reducing inflammation significantly.

Keywords: Anti-inflammatory, Ginger Oil, Inflammatory Process, Natural Remedy, Therapeutic Potential, Zingiber Officinale

1. INTRODUCTION

Ginger (scientific name *Zingiber officinale* Roscoe), is a perennial herb that is used for traditional purposes in the form of fresh paste, ginger tea, dried powder and persevered slices (Altman *et al.*, 2001). Ginger is used as a flavoring agent as well as medical purposes, including ayurvedic, tibbe-e-unani, allopathic, aromatherapy, and household remedies. Ginger oil has antibacterial, antiviral, and

antifungal properties. The rhizomes have a potent aroma used as a seasoning and as a medical agent. Ginger possesses pharmacological effects like antioxidant, anti-inflammatory, anti-nociceptive, anti-emetic, anti-carcinogenic, anti-apoptotic, and immune-modulating effects. Ginger is enriched with various bioactive phenolics, like gingerols, paradols, shogaols, and zingerones (Butt and Sultan 2011). Terpenes and phenolic compounds are the phytochemical groups of ginger. Nonvolatile component, phenolic chemicals in ginger exhibits pharmacological effects. Like gingerols when heated make gingerols, gingerol derivatives 6, 8, and 10, and the homologous shogaol and zingerone family (Altman *et al.*, 2001).

Shagaol, paradols, and gingerol are responsible for its strong flavor and aroma. The volatile components of ginger are terpenes including sesquiterpenes (responsible for fragrant smell) and monoterpenes (ginger flavor). Gingerol (6-gingerol) is responsible for the pungent fragrance and the spiciness of ginger, in rhizomes varying from 104-965 micrograms per gram. Other gingerols including 4-gingerol, 8-gingerol, 10-gingerol and 12-gingerol are present in low concentration, when heated they turned into shagaols (gives strong and spicy aroma). According to Butt and Sultan (2011), shogaols with 6-shogaols derived from dried ginger being the dehydration product. Shogaols possess hydration properties via conversion process. The hydrogenation of shogaols turn into paradols that show medicinal properties. Nigam *et al.* (2009). Ginger works to treat many disorders, like digestive disorders, arthritis, diabetes mellitus, cancer, hypertension, cardiovascular disease (CVD), and atherosclerosis. Ginger prevents aging associated disorder, owing to its antioxidant properties (Dugasani *et al.*, 2010).

Singletary (2010) reported that the plant exhibited efficacy in mitigating the occurrence of emesis and nausea in the context of pregnancy, chemotherapy, and surgical procedures. Ginger can enhance the physiological parameters, including metabolism, blood glucose levels, blood pressure, blood lipids, and glycemic index (Ammon and Wahl, 1991). When fresh ginger was dried, charred or roasted the concentrations of 6-shogaols increased while concentrations of 6,8 and 10GN decreased. Fresh ginger rhizomes are a source of 6SG. High-performance liquid chromatography coupled with a sensitive time-of-flight mass spectrometry approach allows for the quantification of chemicals related to ginger in fresh and dried ginger as well as hot water extract (Mughal, 2019). The main objectives were to make ginger oil using Soxhlet method, ginger oil is antioxidant and phytochemical, ginger exhibits anti-inflammatory properties.

2. MATERIALS AND METHODS

2.1 Area of Research

Department of Nutritional Sciences, Government College University Faisalabad, conducted research.

2.2 Preparation of Ginger Oil from Ginger

They get required quantity of the dried ginger rhizomes from Jhang Bazar Faisalabad and make sure there was no contaminations and moisture. After washing, ginger was sliced, dried, crushed and screened. Soxhlet apparatus was set accordingly. Methanol was used as solvent for extracting ginger oil. The extraction of oil was done under optimum extracting conditions with temperature ranging between 70°C to 80°C. Extraction times ranging from one to one and half hours for tests (Chakraborty *et al.*, 2012).

The weighted ginger rhizomes were added into the Soxhlet apparatus then solvent was added hence the process started by heating. The apparatus continuously cycled the solvent with the ginger for several hours (6-8 hours) or until there's no oil left to extract in the condenser (Funk *et al.*, 2009). Pure ginger oil was obtained by removing the solvent from extract by rotary evaporator. The purified ginger oil was then stored into storage vials or containers (Ammon and Wahl, 1991).

2.3 Chemical Characterization of Ginger Oil

2.3.1 Proximate Composition

The content of moisture, protein, crude fat, ash, and crude fiber was determined by proximate composition of sample. The carbohydrate content was determined by 100 minus the total percentage

of ash, protein, fat and fiber (AOAC, 1999). The energy value was identified by multiplying the physiological values of carbohydrates, protein and fat with factors accordance to AOAC (1999) (Chakraborty *et al.*, 2012).

Physio-Chemical Properties of Ginger Oil

Refractive Index Analysis:

The optical properties of ginger oil were recorded by determining the refractive index. The primary instrument was refractometer Ozgoli *et al.* (2009), the refractometer was set, employing a standard reference liquid. Few drops of ginger oil were poured on the refractometer's prism, ensuring the lid is fully closed. Consequently, observe the refractive index by eyes.

Specific Gravity Determination:

To identify the density of Ginger oil relative to water (Grzanna *et al.*, 2005). The analytical balance Viljoen *et al.* (2014) and ginger oil sample were used. Weight of clean gravity bottle (m1) was determined. Weight of required quantity of ginger oil (m2) was measured. The specific gravity value can be obtained by this formula:

$$\text{Specific Gravity (SG)} = (m2 - m1) / [(m2 - m1) - (m3 - m1)]$$

In this, weight of bottle with water is m3.

Moisture Content Analysis (%):

For assessing quality, the moisture in the ginger oil sample was identified (Habib *et al.*, 2008). During drying process, moisture present in ginger is equal to its weight lost. For this analysis, an analytical balance, a drying oven, an aluminum or glass dish, and the ginger oil sample were used. The procedure started by the weight of clean, dry aluminum dish (W1). Oil was added and measured (W2). Then this dish was placed into drying oven at 105°C for 2 hours. After cooling it was reweighed (W3).

The moisture content percentage was identified by using the formula:

$$\text{Moisture Content (\%)} = [(W2 - W3) / (W2 - W1)] \times 100$$

Mineral Content

Ammon and Wahl (1991) proposed the method to know the contents of calcium, iron, zinc, magnesium, manganese and chromium in ginger powder by Atomic Absorption Spectrophotometer

Total Phenolic Content Assay

Total Phenolic Content Assay procedure was conducted regulating protocols within the field (Gupta *et al.*, 2016). Suitable solvent was used to make stock solution of Ginger oil. A known quantity of standard Gallic acid solution was employed to make a measuring curve. 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate solution was kept in separate tubes with 0.1 mL of Ginger oil stock solution in them. Absorption was recorded at 765 nm utilizing a UV-Vis spectrophotometer for 30 minutes with incubating period in dark at room temperature. The experiment was repeated for 3 three times, the results were observed in mg of Gallic acid equivalents per gram of Ginger oil (mg GAE/g).

$$\text{TPC (mg GAE/g)} = A \times D \times V / W$$

In this concentration of Gallic acid standard solution is A, dilution factor as D, final volume of sample (in mL) is represented by V, the weight of sample (in g) used in the assay is W.

Assessment of Phytochemical Components in Ginger Oil

Assessing the phytochemical components in the ginger oil, such as saponin, HCN, phytin, oxalate and tannin. Phytochemical components of ginger oil were assessed by analytical methodologies. (Chakraborty *et al.*, 2012)

Analysis of Saponin Content: When oil sample was added in distilled water and heated well, the saponin content was recorded. Distilled water was mixed with required amount of oil to be used. The

formation and stability of solution tells the concentration of saponin within sample (Grzanna *et al.*, 2005).

Quantification of HCN Content: When HCL and ammonium chloride was mixed with ginger oil, this is called colorimetric technique. This solution was mixed with sodium picrate reagent to recorded the absorption of colored solution using spectrophotometer. This absorption value then compared to predefined standard curve to know the HCN content within ginger oil (Chrubasik *et al.*, 2005).

Evaluation of Phytin Content: Solution made by mixing sodium hydroxide and ferric chloride with ginger oil. After particular reaction time, absorbance value of resultant coloured solution was recorded by spectrophotometer was compared to a standard curve to assess the content of phytin in the ginger oil via colorimetric technique. (Chrubasik *et al.*, 2005).

Quantification of Oxalate Content: To know the content of oxalate via gravimetric technique, the precipitation of calcium oxalate, calcium chloride (CaCl₂) and ammonium oxalate solution (NH₄)₂C₂O₄ was supported was reacted with ginger oil. The solution thus followed filtration, drying and weighing. The dried precipitate shows the content of oxalate content in the oil. (Funk *et al.*, 2009).

Assessment of Tannin Content: Folin-Denis reagent and sodium carbonate was mixed with ginger oil to form ginger oil solution. The absorbance of the resulting solution was known by using spectrophotometer. Later compared with standard curve to get tannin content in ginger oil (Chakraborty *et al.*, 2012).

These analytical processes show the phytochemical composition of ginger oil, subsequently, shows the potential therapeutic properties including inflammatory responses (Funk *et al.*, 2009).

Antioxidants Assay of Gingerol from Ginger Oil

Gingerol exhibits preventive potential, antioxidant and anti-inflammatory properties. To examine the constituent parts of ginger oil. The infrared spectroscopic examination of ginger oil was carried out (Funk *et al.*, 2009).

Analysis of Antioxidants

Antioxidants was calculated and represented as mg Quercetin equivalents/g (mg QE/g).

DPPH Assay: DPPH (2,2-diphenyl-1-picryl-hydrazyl) has the ability to combine with free radicals that helps to understand the level of antioxidant activity in both test sample and in standard methanol extract. Methanol served as medium for making the test sample solution, consisted of diluted test sample components. Quantities varying from 5 × 10⁴ to 4 × 10³ mg/ml of Gallic acid acts as standard. 0.135 moles per liter of DPPH was prepared in methanol. Independent test was conducted on standard solution, 2ml of this DPPH solution was mixed with 2ml of sample solutions I, varying in concentration from 1mg/ml to 8 mg/ml. Optical density of gingerol was recorded in every solution, after placing them in the dark for 30 mins (Black & Herring, 2010).

Refractive index, color, acid value, and peroxide value tells the quality of ginger oil.

Absorbance of Control – Absorbance of Sample/Absorbance of Sample x 100

FRAP Assay

According to Benzie and Strain (1996) the reduction of (Fe³⁺) ferricyanide in stoichiometric excess relative to the antioxidants. The reduction capacity of extracts as established parameters outlined by Imo (2019). A mixture of 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% of potassium ferricyanide (pH 6.6) was added to the 1mL of EEG or EET in ethanol. For 20 minutes the mixture was left in ultrasound water bath having 50°C. The mixture was centrifuged (2000 rpm) for 10 minutes then mixed with 2.5 mL of 10% trichloroacetic acid. 2.5 mL of supernatant was mixed with 0.5 mL of 0.1 % ferric chloride and 2.5 mL of distilled water. The colored solution was recorded at 700 nm when compared with standard with the help of ultraviolet spectrophotometer. Ascorbic acid act as quality control. Decreasing the iron was the capacity of sample when comparing with gold standard. Higher absorption exhibits greater reduction capacity. (Kustriyanti, 2019)

FRAP Value = Absorbance Sample + Frap Reagent – Absorbance (FRAP Reagent)

Anti-inflammatory Potential

In vitro anti-inflammatory potential of gingerol was assessed by two methods. Its impacts on inflammatory cytokines like IL-8, IL-1 and TNF-, including NO₂ production assay and the enzyme-linked immunosorbent assay (ELISA) (Mughal, 2019).

Nitric Oxide Production

Griess reaction was used to measure the presence of NO₂ indicating inflammation in the body. Inflamed cells were incubated on the 60 mm dishes. The medium was changed with DMEM after 24 hours of incubation. Then pre incubation was done on gingerol for 30 minutes. Then 0.2 g/ml of lipopolysaccharide was reacted with plate, and incubated for 8 hours at 37°C. The 96-well microplate was incubated for 15 minutes before Griess reagent was added to the media. The standard curve made due to the addition of NaNO₂ to the microplate observed by microplate reader were used to compute concentrations (Al-Nahain *et al.*, 2014).

High-Performance Liquid Chromatography (HPLC) Analysis

Ginger oil's chemical composition was identified by HPLC Analysis. HPLC employed with high pressure pump, injector, column oven, UV-Vis detector and a C18 reverse-phase column. Appropriate solvent was used to make sure its compatibility with the HPLC system. A gradient elution method was used having mobile phase consisting of acetonitrile and water having formic acid as an ion-pairing agent. Flow rate, temperature and wavelength nm was adjusted. The standard solution of ginger oil identifies the concentration of each compound within ginger oil. 3 times the sample was recorded to represent mean \pm standard deviation. The chemical composition of ginger oil helps a lot to understand its therapeutic and anti-inflammatory properties, enhancing significant value to this research (Al-Nahain *et al.*, 2014).

Ginger rhizome and callus extracts were diluted in HPLC-grade chloroform and 1:1 methanol for analysis. Make 20 mL V of extracts with methanol, 1mg/mL solution was created. After filtering, concurrent measurement of 6-gingerol, 6-shogaol, and Gallic acid were obtained when HPLC analysis was done. For standard solution, methanol was mixed with 6-gingerol and 6-shogaol. Until usage, 4°C is managed for standard solution (Abdi *et al.*, 2021).

To identify the content of 6-gingerol, 6-shogaol, and Gallic acid, ginger rhizomes and callus extract go through qualitative and quantitative tests by HPLC. When comparing the chromatograms, it was observed that rhizomes extract exhibits compounds 6-gingerol and 6-shogaol while callus extracts showed none of these compounds, as depicted in Figure 3. The concentration of 6-shogaol was lesser than 6-gingerol. PE extract of rhizome showed more 6-gingerol whereas CM extract showed largest concentration of 6-shogaol (Mughal, 2019).

Biological remedies are common for inflammatory maladies, involving the targeted suppression of certain elements within the inflammatory cascade, such as cytokines (Viljoen *et al.*, 2014). In this work, it was observed that the extracts of ginger rhizome and callus, specifically the petroleum ether (PE) and chloroform-methanol (CM) extracts, had a dose-dependent inhibitory effect on the synthesis of pro-inflammatory cytokines (TNF-, IL-1, and IL-6) by macrophages activated with lipopolysaccharide (LPS). The production of cytokines decreased. The suppressive effects of rhizome PE and CM extracts were recorded same. While, rhizome extracts show less activity levels than callus extracts. On enhancing the concentration of extract to 100g/ml, improvement was seen in the inhibitory capacity in contrast with TNF production for both PE and CM callus extract.

Statistical Analysis:

By SPSS, the statistical analysis of data was done and make tables to find the means. (Steel *et al.*, 1997) reported that the degree of significance was determined with the help of the analysis of variance approach (ANOVA).

3. RESULTS AND DISCUSSION

Proximate Composition

The modifier level of the sample is 6.32% showing that ashing might take less time. The chemicals composition of ginger is given in the table

Ginger	Moisture	Ash	Fat	Fiber	Proteins	CHO	Energy /Kcal
	94.40±0.033	3.70±0.013	3.70±0.152	7.30±0.056	2.65±0.041	74.49±0.195	338.80±0.653

Table 1: Proximate-composition of ginger and its products (%/100gm)

his suggests that ginger rhizome powder would have a storage life of extended and that microbial action-induced degradation was confined (Black & Herring, 2010).

Physicochemical Composition

The Physicochemical features covers the identity and attributes of oils. According to the study by Supu *et al.* (2019) identified the identity characters such as its specific gravity, its refractive index, its moisture content, its iodine value, its saponification value, and its fatty acid composition Quality features include color, free fatty acid, acid value, and peroxide value the oil possess moisture and high concentrated red pigment which gives off red color of value of 38 units and this value decrease by carrying the further tests.

Parameters Results	Mean ± SD
Refractive index	1.47 ± 0.11
Specific gravity	0.85 ± 0.21
Moisture content (%)	0.52 ± 0.20
Yield (%)	2.0 ± 0.70

Table: Physicochemical parameters of ginger oil.

The yield from ginger rhizome oil is only 2% which is very low so it is not a preferable source. The study shows that the ginger oil contains all the essential fatty acids for the body and holds the properties of inhibiting microorganism (Grzanna *et al.*, 2005).

Mineral Content

The mineral composition of substances can vary due to various factors like what kind it is, what variety it is, under what agronomic conditions, how they are processed and stored (Shieh *et al.*, 2015). The young ginger has higher levels of minerals than adult ginger.

Table: Mineral composition of ginger

Mineral	Ca	Mg	Mn	Fe	Zn
Concentration (mg/100g)	9.66±1.16	195.40±0.54	21.81±0.25	33.10±1.3	1.89±0.19

Total Phenolic Content (TPC) Assay

The understanding of the chemical composition of the investigated ginger oil, is given by the Total Phenolic Content (TPC) assay, exhibiting a significant phenolic content averaging around 35.2 mg gallic acid equivalents (GAE) per gram. This high level of phenolic compound plays a part in the property of antioxidation, in the therapeutic effects of ginger oil, specifically in mitigating oxidative stress and inflammation. The TPC helps in apprehending the anti-inflammatory property of ginger oil (Grzanna *et al.*, 2005).

Determination of phytochemical composition of Ginger Oil

Compound	Concentration g/mg
Saponin	3.00±0.05
HON	0.70±1.02
Phytin	0.26 ± 0.02
Oxalate	0.24 ± 0.01
Tannin	0.02 ± 0.01

According to the above table in ginger oil the saponin level is high (3.01/100mg) and the tannin content is low (0.03 mg/100g) which shows a difference in value of saponin level in rhizome powder of 3.85 mg/100g with the research done by Roufogalis *et al.* (2014). But the high level of saponin confirm the previous researches by Ozgoli *et al.* (2009) indicating potential for gastrointestinal health benefits and nutrition absorption.

The number of cyanogenic glycosides found In *Congronema latifolia* (7.07 mg/100 g) is higher than found in ginger (2.81 mg/100 g) by Murakami *et al.* (1999).

HPLC analysis

The study by Kizhakkayil and Sasikumar (2012), in which High-Performance Liquid Chromatography (HPLC), gives consistent calluses of 6-gonewild and 6-shogaols. Both having high concentration but later is less as compared to prior one. These two compounds are absent in callus during callus cultivation due to differentiation. The level of 6-gingerol is significantly lower in callus cultured and micro-propagated plants in comparison with the plants grown by conventional cultivation methods (Liu *et al.* 2006)

Further analysis of ginger rhizome and callus extracts showed the existence of Gallic acid in the chloroform-methanol (CM) extracts while it was not present in petroleum ether. A prominent phenolic acid, gallic acid, is found in ginger (Ghasemzadeh *et al.*, 2011). The amount of gallic acid depend on the type of elicitor in the callus CM extracts.

Compound	Sample	Concentration	Presence in PE Extract	Presence in CM Extract
6-gingerol	Ginger Oil	Highest	Not detected	Present
6-shogaol	Ginger Oil	Lower than 6-gingerol	Not detected	Present
6-gingerol	Ginger Callus	Lower (vs. ginger rhizome)	Not detected	Present
6-gingerol	Micro-propagated Rhizome	Lower (vs. traditionally produced rhizome)	Not detected	Present

Table: HPLC analysis of ginger rhizome and untreated callus extracts.

Ferric Reducing Antioxidant Power

The Ferric Reducing Antioxidant Power (FRAP) an electrochemical titration-based test that checks the ability of an anti-oxidant Fe^{3+} ion complex to a colored Fe^{2+} complex in acidic conditions. Which includes measuring absorbance at 593 nm and forms a curve. FRAP values for ginger oil are commonly expressed in millimoles of Trolox equivalents per gram (mM TE/g) or per milliliter (mM TE/mL) of ginger oil (Sakr *et al.*, 2011).

Table presents FRAP values for fresh and dried ginger oil samples, indicating antioxidant capacity.

Sample ID.	Absorbance	FRAP Value (in mM TE/g or mM TE/mL)
Ginger Oil A (Fresh Ginger)	0.725	14.5
Ginger Oil B (Dried Ginger)	0.590	11.2

Antioxidant potential is understood by FRAP values which are recorded to be different in different parts of gingers, processing methods and storage conditions. The FRAP values are compared with

Trolox (antioxidants) to assess its antioxidant capacity. The impact of bioactive components, such as gingerol, on antioxidant capacity is also considered. (Sakr *et al.*, 2011).

DPPH Scavenging Activity of Ginger Oil (μL)

The DPPH test evaluates the antioxidant properties by exposing the substance to ethanol or methanol solutions by recording its capacity to scavenge free radicals. (Viljoen *et al.*, 2014). DPPH, is highly stable showing no reactivity towards other ions or electrons, can accept hydrogen ions or electrons. In this study, different parts of ginger plant were tested for their DPPH values, influenced by both portion and solvent factors. DPPH is soluble in methanol and ethanol, with characteristic absorption peaks at 517 nm. The spectrophotometric determination of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical at 515-517 nm discloses the scavenging capacity of the substance radical NY inducing color change in DPPH. The DPPH test is used widely due to its simplicity and sensitivity, employing a dark purple stable radical. Antioxidants reduce DPPH levels, transforming it into a stable diamagnetic molecule. According to Basma *et al.* (2011), the evaluation was done by DPPH Assay. The reduced quantity of DPPH present in the specimen due to the efficiency of antioxidants

NO₂ (Nitrite Ion) Production Assay:

Inflammation is closely linked to malignancies, with tumors often exhibiting high levels of the nitric oxide radical (NO), which can impact cancer prognosis. (Prior *et al.*, 2005). The presence of inflammation can be verified by the high level of nitric oxide levels, but under extreme stress, it can oxidize to form nitrite ions (NO₂). The table highlights gingerol's different doses inhibitory effect on NO₂ production in LPS RAW 264.7 cells, demonstrating its potential anti-inflammatory activity (Kiuchi *et al.*, 1992).

Table 4.3 Shows the potential of gingerol to half the production of nitrite ions.

Treatment doses	Ginger oil (μL) Quantity/dose	NO ₂ Production
T1	LPS (0.2 $\mu\text{g/ml}$)	6.4657 \pm 0.2 mM
T2	LPS (0.2 $\mu\text{g/ml}$) + Gingerol (15 μM)	4.4946 \pm 0.3 mM
T3	LPS (0.2 $\mu\text{g/ml}$) + Gingerol (25 μM)	3.4 \pm 0.6 mM
T4	LPS (0.2 $\mu\text{g/ml}$) + Gingerol (35 μM)	0.701 \pm 0.7mM

Ginger Oil Effect on Over-expression of COX-2

The enzyme cyclooxygenase (COX) is the target for medication to reduce inflammation by converting arachidonic acid into inflammatory prostaglandins. Inflammatory processes activate the cyclooxygenase-2 (COX-2) enzyme, which contributes in the formation of inflammatory mediators, including prostaglandin (PGE₂) and these medications are suppressed by stopping the COX (Habib *et al.*, 2008).

4. SUMMARY

The phytochemical in ginger oil shows dual benefits of regulating body's antioxidant system and searching free radicals. Its nutraceutical qualities encloses antioxidant, anti-inflammatory, gastroprotective, immune-modulatory, neuroprotective, cardioprotective, chemo-preventive, and antidiabetic agents. Ginger have been investigated clinically by nations for the benefit of human and animal health. Quantification through HPLC reveals varying gingerol content in different parts of ginger, with leaves exhibiting the highest (4.19 msg/g). It was determined that the essential ginger oils dissolves easily in ethanol extract than in methanol and water. Ginger contains many compounds that have similar chemical structures like Gingerol, shogaol and other chemical compounds. The 6-shogaol is the most significant one because it possesses antioxidant and anti-inflammatory properties due to the presence of an alpha, beta-unsaturated ketone moiety in ginger. The Soxhlet extraction method proves to be effective for treating bacterial illnesses. The study reveals that all extracts except water pursue antibacterial effects. The chemical compounds like zingiberene and sesquiterpenoids exhibits antibacterial and inhibitory functions. The findings conclude that the leaves of *E. elatior*

might perform as a source of antioxidants. However, it shows variations in radical scavenging by extracts of ginger leaves and rhizome. The results of the FRAP technique and DPPH strategy shows that the sample act as an antioxidant.

Ginger where use as a condiment it also helps in the treatment of cardiovascular problems, diabetes mellitus and gastrointestinal health. Its use had been historically safe and even encouraging for future use.

5. REFERENCES:

1. Abdi, T., and Abdul Wahab, N., (2021). Ginger (*Zingiber officinale Roscoe*) Extract protects the heart against inflammation and fibrosis in diabetic rats. *Canadian Journal of Diabetes*, 45(3), 220–227.
2. Abdullahi A, Khairulmazmi A, Yasmeen S, Ismail IS, Norhayu A, Sulaiman MR, Ahmed OH, Ismail MR. (2020). Phytochemical profiling and antimicrobial activity of ginger (*Zingiber Officinale*) essential oils against important phytopathogens. *Arabian Journal of Chemistry*, 13(11), 8012-25.
3. Al-Nahain, Abdullah, Jahan, Rownak, & Rahmatullah, Mohammed. (2014). Zingiber officinale: A Potential Plant against Rheumatoid Arthritis. *Arthritis*, 2014, 159089.
4. Altman, Roy D., and Krishna K., (2001). Effects of a ginger extract on knee pain in patients with osteoarthritis. *Arthritis & Rheumatism*, 44(11), 2531-2538.
5. Ammon, Herbert P., & Wahl, Marco A. (1991). Pharmacology of Curcuma longa. *Planta Medica*, 57(1), 1-7.
6. Black, C. D., & Herring, M. P. (2010). Ginger (*Zingiber officinale*) reduces muscle pain caused by eccentric exercise. *The Journal of Pain*, 11 (9), 894-903.
7. Butt, M. S., & Sultan, M. T. (2011). Ginger and its health claims: molecular aspects. *Critical Reviews in Food Science and Nutrition*, 51 (5), 383–393.
8. Chakraborty, D., Mukherjee, A., Sikdar, S., Paul, A., Ghosh, S., & Khuda-Bukhsh, A. R. (2012). [6]-Gingerol isolated from ginger attenuates sodium arsenite induced oxidative stress and plays a corrective role in improving insulin signaling in mice. *Toxicology Letters*, 210 (1), 34-43.
9. Chrubasik, S., Pittler, M. H., & Roufogalis, B. D. (2005). Zingiberis rhizoma: A comprehensive review on the ginger effect and efficacy profiles. *Phytomedicine*, 12 (9), 684-701.
10. Dugasani, Satyanarayana, et al. (2010). Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol, and [6]-shogaol. *Journal of Ethnopharmacology*, 127(2), 515–520.
11. Funk, J. L., Frye, J. B., Oyarzo, J. N., Kuscuoglu, N., Wilson, J., McCaffrey, G., ... & Timmermann, B. N. (2009). Efficacy and mechanism of action of turmeric supplements in the treatment of experimental arthritis. *Arthritis & Rheumatism*, 60 (2), 323-331.
12. Gupta, R., Singh, P. K., Singh, R., & Singh, R. L. (2016). Pharmacological activities of Zingiber Officinale (ginger) and its active ingredients: A review. *International Journal of Innovation Science and Research*, 4, 1–18.
13. Grzanna, R., Lindmark, L., & Frondoza, C. G. (2005). Ginger—an herbal medicinal product with broad anti-inflammatory actions. *Journal of Medicinal Food*, 8 (2), 125–132.
14. Habib, H. M., Liju, S. H., Thampan, R. V., Kuttan, R., & Kuttan, G. (2008). Ginger extract (*Zingiber Officinale*) has anti-cancer and anti-inflammatory effects on ethionine-induced hepatoma rats. *Clinics*, 63(6).
15. Imo, C. (2019). Medicinal properties of ginger and garlic: A Review. *Current Trends in Biomedical Engineering & Biosciences*, 18 (2).
16. Jeena, K., Liju, V. B., & Kuttan, R. (2015). Antioxidant, anti-inflammatory, and antinociceptive activities of essential oil from ginger. *Indian Journal of Physiology and Pharmacology*, 12 (Source).
17. Kiuchi, Masataka H., Sun, Wenkui, & Cheng, Qi. (1992). Pharmacological uses and health benefits of ginger (*Zingiber Officinale*) in traditional Asian and ancient Chinese medicine, and modern practice. *Notulae Scientia Biologicae*, 3, 309–319.

18. Kizhakkayil, J., & Sasikumar, B. (2012). Characterization of ginger (*Zingiber Officinale Rosce*) germplasm based on volatile and non-volatile components. *African Journal of Biotechnology*, 11 (4), 777-786.
19. Kustriyanti, D., & Putri, A. A. (2019). Ginger and lemon aromatherapy to relieve nausea and vomiting in pregnancy. *Jurnal Keperawatan Soedirman*, 14 (1), 15.
20. Liu, C. T., Jia, R. Z., Yang, W. R., Wang, T., Wei, G. T., & Yi, J. L. (2006). Antidiabetic effect of garlic oil but not diallyl disulfide in rats with streptozotocin-induced diabetes. *Food and Chemical Toxicology*, 44(8), 1377–1384.
21. Lua, P. L., Salihah, N., & Mazlan, N. (2015). Effects of inhaled ginger aromatherapy on chemotherapy-induced nausea and vomiting and health-related quality of life in women with breast cancer. *Complementary Therapies in Medicine*, 23 (3), 396-404.
22. Mukherjee, S., Mandal, N., Dey, A., & Mondal, B. (2014). An Approach towards optimization of the extraction of polyphenolic antioxidants from ginger (*Zingiber Officinale*). *Journal of Food Science and Technology*, 51 (11), 3301–3308.
23. Mughal MH. (2019). 6-gingerol and shogaol; a comprehensive strategy against various maladies. *Significances of Bioengineering and Bioscience*, 3, 1-9.
24. Murakami, A., Takahashi, M., Jiwajinda, S., Koshimizu, K., & Ohigashi, H. (1999). Identification of zerumbone in zingiber zerumbet smith as a potent inhibitor of 12-O-tetradecanoylphorbol-13-acetate-induced epstein-barr virus activation. *Biosci. Biotechnol. Biochem.*, 63, 1811–1812.
25. Nigam, N., Bhui, K., Prasad, S., George, J., & Shukla, Y. (2009). [6]-Gingerol induces reactive oxygen species regulated mitochondrial cell death pathway in human epidermoid carcinoma A431 cells. *Chemico-Biological Interactions*, 181 (1), 77-84.
26. Oforma, C. C., Udourioh, G. A., & Ojinnaka, C. M. (2019). Characterization of essential oils and fatty acids composition of stored ginger (*Zingiber Officinale Roscoe*). *Journal of Applied Sciences and Environmental Management*, 23 (12), 2231-2238.
27. Ogbuwu, I., Jiwuba, P., Ezeokeke, C., Uchegbu, M., Okoli, I., & Iloeje, M. (2014). Evaluation of phytochemical and nutritional composition of ginger rhizome powder. *International Journal of Agricultural and Rural Development*, 17 (1), 1663-1670.
28. Ojulari, L. S., Olatubosun, O. T., Okesina, K. B., & Owoyele, B. V. (2014). The effect of *zingiber officinale* (ginger) extract on blood pressure and heart rate in healthy humans. *IOSR Journal of Dental and Medical Sciences*, 13 (10), 76–78.
29. Ozgoli, G., Mojab, F., Gharabagh, M. S., et al. (2009). Comparison of effects of ginger, mefenamic acid, and ibuprofen on pain in women with primary dysmenorrhea. *Journal of Alternative and Complementary Medicine*, 15(2), 129-132.
30. Roufogalis, Basil D., et al. (2014). *Zingiber officinale* (Ginger): A future outlook on its potential in prevention and treatment of diabetes and prediabetic states. *New Journal of Science*, 2014.
31. Sakr, S. A., Badawy, G. M., & (2011). Effect of ginger (*Zingiber Officinale R.*) on metiram-inhibited spermatogenesis and induced apoptosis in albino mice. *Journal of Applied Pharmaceutical Science*, 5(6), 131–136.
32. Shieh, Y.-H., Huang, H.-M., Wang, C.-C., Lee, C.-C., Fan, C.-K., & Lee, Y.-L. (2015). Zerumbone enhances the Th1 response and ameliorates ovalbumin-induced th2 responses and airway inflammation in mice. *Int. Immunopharmacol.*, 24, 383–391.
33. Singletary, K. (2010). Ginger: An overview of health benefits. *Nutrition Today*, 45 (4), 171-183.
34. Sueishi, Y., Masamoto, H., Kotake, Y., & Kinoshita, M. (2019). Heat treatments of ginger root modify but not diminish its antioxidant activity as measured with the MULTIS method. *Journal of Clinical Biochemistry and Nutrition*, 65(2), 143–147.
35. Supu, Rahmadona Dewi, Diantini, Aris, & Levita, Jullieta. (2019). Red ginger (*Zingiber Officinale Var. Rubrum*): Its chemical constituents, pharmacological activities, and safety. *Fitofarmaka: Jurnal Ilmiah Farmasi*, 1, 23–29.
36. Vijayaraghavan, K., Rajkumar, J., & Seyed, M. A. (2018). Phytochemical screening, free radical scavenging, and antimicrobial potential of *Chromolaena odorata* leaf extracts against pathogenic

- bacterium in wound infections—a multispectrum perspective. *Biocatalysis and Agricultural Biotechnology*, 15, 103-112.
37. Viljoen, E., Crowther, C. A., Middleton, P., & Stewart, A. W. (2014). A systematic review and meta-analysis of the effect and safety of ginger in the treatment of pregnancy-associated nausea and vomiting. *Nutrition Journal*, 13, 20.
 38. Yeh, H.-Y., *et al.* (2014). Bioactive components analysis of two various gingers (*Zingiber Officinale Roscoe*) and antioxidant effect of ginger extracts. *LWT - Food Science and Technology*, Volume (1), 329–334.
 39. Zadeh, J. B., & Kor, N. M. (2014). Physiological and pharmaceutical effects of Ginger (*Zingiber Officinale Roscoe*) as a valuable medicinal plant. *European Journal of Experimental Biology*, 4(1), 87-90.