



## A Study of Antibacterial and Antioxidant Activities of Zingiber Officinale (ROSC) Extracts

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### KEYWORDS

Ginger (Zingiber officinale), methanol extract, DPPH radical, HPLC, Medicinal plant, Spice, rhizome.

### ABSTRACT:

Ginger (Zingiber officinale), a member of the Zingiberaceae family, which natively belongs mainly to the Southeast Asia region. It is a perennial creeping plant with long leaves, yellow-green blooms, and a thick tuberous rhizome. This particular storage root has a strong flavour. One of the most adaptable medicinal plants with a broad range of biological activity, ginger has been used medicinally for more than 2000 years. Since ancient times, ginger has been used in Ayurvedic and Chinese medicine to treat a variety of conditions, including menstrual irregularities, cancer, osteoarthritis, food poisoning, nausea, inflammation, coughing, and colds. In addition to these, it demonstrates antioxidant and antibacterial effects. Ginger has therapeutic qualities that are paradol, shogol, and gingerol. etc. These results also demonstrated the existence of many phytochemical elements whose biological activity may have significant therapeutic value. On a scale of 1 to 4, the colour intensity represents the outcome of the phytochemical analysis in Zingiber officinale extract (+, ++, +++, ++++). The table shows the DPPH radical activity of several solvents of Zingiber officinale rhizome extracts. The methanol and water extracts of Zingiber officinale at 400 µg/ml exhibited about 98.602±0.026 of methanol and 96.148±0.117 water extracts. The DPPH activity was found comparable to ascorbic acid (std) which showed a DPPH scavenging activity of 99.472±0.026. The concentration of the sample at which the inhibition percentage reaches 50% is IC<sub>50</sub>. The lower the IC<sub>50</sub> higher the antioxidant potential. Methanol extracts showed better antioxidant potential when compared with water extracts using the DPPH scavenging activity method. Zingiber officinale in water extract shows better antioxidant potential activity of 6.220±0.091 and 9.065±0.128 µg/ml in methanol extracts.

For the examination of antibacterial properties, we used 4 standard species of bacteria Escherichia coli [MCC 3099], Streptococcus pneumoniae [MCC 2424], Bacillus subtilis [MCC 2010], Klebsiella pneumoniae [MCC 3094]. The MIC (Minimum Inhibitory Concentration) was performed for methanol extraction of Zingiber officinale's rhizome by ELISA technique. The presence of alkaloids, cardiac glycosides, flavonoids, saponins, etc. was also examined during phytochemical analysis. The presence of antioxidants has been determined by DPPH radical scavenging capacity, Antioxidant potential IC<sub>50</sub>, and chromatogram of High-Pressure Liquid Chromatography Standard. The finding concluded that the methanol extract of Ginger expressed antimicrobial potential against the above-mentioned bacteria species. So Ginger can be safely used as a conventional medicinal source against bacteria.



## INTRODUCTION

Medicinal plants have been traditionally used to cure different pathological conditions. As per reference to World Health Organization (W.H.O.), about 80% of the world's population mostly relies on conventional treatment methods that use plant extracts and their active ingredients [18]. Also, traditional healthcare systems rely mainly on medicinal plants and their extracts to treat a wide variety of ailments. Medicinal plants efficacy comes from biochemicals (also known as bio-nutrients or phytochemicals or secondary metabolites) constituents that have a clear physiological effect on the human body [3]. A broad range of phytochemicals (such as, alkaloids, chlorogenic acid, gallic acid, hydroquinone, tannins, terpenoids, flavonoids, and other secondary metabolites) have been identified from the extract from bark, leaf, flower, and fruit of different plants [1].

Ginger (*Zingiber officinale* Roscoe) is a member of the family Zingiberaceae, which is assumed to have first been grown in the region of China and subsequently moved in another part of the South East Asia and other continents [8]. Ginger is a significant plant with numerous therapeutic, folk medical, and nutritional benefits [4]. Ginger has been used in Chinese and Ayurvedic medicine to treat nausea, diarrhoea, and stomach discomfort as well as cardiac problems [18]. It is also used to mask the taste of medications; it encourages the production of bile from the gall bladder, reduces arthritis-related joint discomfort, helps treat heart and lung problems, relieves cough and cold symptoms, and treats throat infections [13-12]. Furthermore, the pharmacological value of ginger has been well established as an active immune modulator by which it inhibits cyclooxygenase-1 and cyclooxygenase-2, which cumulatively reduces prostaglandin production and reduces inflammation [22]. Traditional use and documentation in Ayurvedic literature, evidence indicate that the extract of ginger consists of antioxidant and antibacterial properties [1]. However, the lack of strong scientific evidence makes us investigate the antioxidative capability of ginger extract using the DPPH method. For this purpose, we aimed to investigate the presence of phytochemicals in ginger extract using biochemical methods and elaborate on its antioxidant properties. This article helps to stabilize the biomedical value of ginger and might provide an answer to a few open questions that make it a valuable medicinal plant, around the globe [19].

Antioxidant and phytochemical properties of purified rhizome extracts of *Zingiber officinale*, commonly known as ginger, antioxidant activity is important because it helps protect our cells from oxidative damage caused by free radicals. Free radicals are unsteady molecules that can harm cells and play a role in the emergence of a number of diseases. Phytochemicals are bioactive compounds found in plants that have potential health benefits [6].

Several studies have shown that purified rhizome extracts of *Zingiber officinale* possess significant antioxidant activity. These extracts contain various phytochemicals such as gingerols, shogaols, and zingerone, which contribute to their antioxidant properties. Gingerols, in particular, have been identified as the major bioactive compounds responsible for the antioxidant activity in ginger [17-21].

The antioxidant activity of ginger extracts has been evaluated using different methods such as DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, FRAP (Ferric Reducing Antioxidant Power) assay, and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) assay. These assays measure the ability of the extracts to scavenge free radicals and reduce oxidative stress [11].

In addition to their antioxidant activity, purified rhizome extracts of *Zingiber officinale* also contain other phytochemicals such as terpenoids, flavonoids, and phenolic compounds. These phytochemicals have been associated with various health benefits, including anti-inflammatory, anti-cancer, and antimicrobial properties, overall, the antioxidant and phytochemical evaluation of purified rhizome extracts of *Zingiber officinale* indicates its potential as a natural source of antioxidants and bioactive compounds that may have positive effects on human health. Further research is needed to fully understand the mechanisms of action and potential therapeutic applications of these extracts [2].

The present research aimed to study the antibacterial, antioxidant and phytochemical evaluation of the bioactive purified rhizome of *Zingiber officinale* also known as ginger and to evaluate the IC<sub>50</sub> activities of ginger extracts (aqueous extract of methanol as well as water extract) of ginger rhizome and to determine the active constituents to which attributed these activities.

## MATERIALS AND METHODS



## A. Collection and preparation of extract from ginger rhizomes

The rhizomes of ginger were collected from the local market of Noida city, U.P. India. Fresh rhizomes of ginger plants were collected and cleaned in tap water, allowed to air dry, homogenized to a fine powder, and then stored in airtight containers. 10g of powdered rhizomes were added in 25 ml of methanol and water, separately, into a conical flask. Both prepared flasks were placed in a soxhlet extraction for 48 hours. After incubation, extracts were filtering using Wattman No. 1 filter paper. The filtrates were collected in sealed containers. The extracts were heated in a water bath and drained to dryness to produce semi-solid bulk. The dried extract was stored for future phytochemical analysis at 4°C.

## B. Preparation of Extract: Solvents Extract

Above mentioned pulverized material mixed with methanol and distilled water. Filtered it with the help of Whatman paper no.1. Further it was stored overnight on a shaker at 100 rpm. The obtained dry extract was weighed and kept at 4°C in the refrigerator for further use.

## C. Phytochemical Analysis

In order to determine the existence of phytochemical elements such as alkaloids, tannins, steroids, saponins, cardiac glycosides, flavonoid, and phenols, phytochemical analysis was done using methanol and water, as solvents.

### Tests for Alkaloids

In order to investigate the alkaloid content in prepared extract. A few drops of Mayer's reagent were added to one section of the extract, the same quantity of Dragendorff's reagent was added to another section, and the same amount of Wagner's reagent was added to the final component. The presence of the corresponding alkaloids is shown by the cream-colored precipitate, orange precipitate, and brown precipitate [16].

### Tests for Flavonoids

The Shinoda test was performed to estimate the content of flavonoids. Magnesium ribbon and a few drops of strong HCl were added to the extract solution. Flavonoids are

present when a pink or tomato-red colour appears within a few minutes [1].

### Tests for Tannins

The extract was treated with an alcoholic  $\text{FeCl}_3$  reagent. The blue colour indicates the presence of tannins [16].

### Test for Cardiac glycosides

Keller-Kilian test was performed to determine the presence of cardiac glycosides in the prepared extracts. One millilitre of the  $\text{FeCl}_3$  reagent (a combination of one volume of 5%  $\text{FeCl}_3$  solution and 99 volumes of glacial acetic acid) was applied to the raw dry powder form of each rhizome. A few drops of concentrated  $\text{H}_2\text{SO}_4$  were added to this solution. Within a few minutes, a greenish-blue colour shows up, indicating the presence of cardiac glycosides [2].

### Tests for Steroids

Steroid examination was conducted by adding 1gm of extract in a test tube, which was then filled halfway with strong sulfuric acid. The undiluted crude rhizome extract was first dissolved in chloroform (10 ml). The sulphuric acid layer in the test tube displayed yellow with green fluorescence, whereas the super layer in the tube turned red. It demonstrated that steroids were present [14].

### Tests for Saponins

The Frothing test was done to identify the presence of saponins. Each rhizome's coarse dry powder was shaken forcefully with distilled water and then analyzed for the presence of saponins for 10 minutes. No froth shows the lack of saponins, and stable foam greater than 1.5 cm confirms the presence of saponins [16].

### Tests for phenols

Phenol test was conducted by adding 5 drops of 10% ferric chloride and 2 ml of distilled water in to the 1 ml of the extract. The presence of phenols causes the creation of blue or green colour [15].

## D. DPPH Antioxidant Activity of Prepared Extracts



The free radical scavenging DPPH assay was used to determine the antioxidant activity of the prepared extracts. The free radical scavenging DPPH assay is well-established methods which are frequently used to determine the antioxidant properties [11-23]. 5 mL of a 0.004% (w/v) solution of DPPH and 50  $\mu$ L of ginger rhizome extracts with concentrations ranging from around 100 to 400  $\mu$ g/mL will be added. A spectrophotometer set to read at 520 nm will be used to read the mixture after it has been vortexed and incubated for 30 minutes at room temperature in a relatively dark environment. 80% (v/v) methanol makes up the blank. An ascorbic acid (Vitamin C) comparison will be made. There will be three copies of each measurement. The following equation is used to determine the DPPH scavenging effect: DPPH radical scavenging activity (%) =

$$\frac{(\text{Absorbance of control} - \text{Absorbance of the sample})}{\text{Absorbance of control}} \times 100$$

$$= \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Where:

The absorbance of control = Absorbance of DPPH radical + methanol

The absorbance of sample = Absorbance of DPPH radical + sample extract / standard.

## RESULTS AND DISCUSSION

### % yield of ginger extracts

The percentage yield of rhizome extracts of *Zingiber officinale* in different solvents is 1.5% and 1.2 % for methanol and water, respectively.

TABLE 1

The percentage yield of solvent extracts of *Zingiber officinale* (Rhizome)

S.No.	Solvent	Weight of dried extract (g)	Yield (%)	Colour	Consistency
1	Methanol	15	1.5	Dark brown	Paste
2	Water	12	1.2	Light brown	Paste

TABLE II

Phytochemical Screening of Methanol and Water Extracts

S.No.	Phytochemical Components	Names of Reagents Used	Name of Extracts	
			Methanol	Water
1	Alkaloids	Mayers test	++	++
		Dragondroff's test	+	+
		Wagner's test	+	+
2	Glycosides	Keller-kiliani's test	++	-



3	Flavonoids	Shinoda test	++	+
4	Saponins	H <sub>2</sub> SO <sub>4</sub> test	+++	+
5	Steroids	Frothing test	+	+
6	Tannins	Ferric chloride test	+	+
7	Phenols	FeCl <sub>3</sub>	+++	++

**Note** (+++): Strongly positive, (++): positive, (+): Trace, and (–): Not detected

### Phytochemical Analysis of Ginger Extracts

It shows saponins and phenols were maximum and Tannins were in trace amount in methanol extraction, while alkaloids and phenol were maximum. Glycosides were not detected in water extraction. Only alkaloids remained the same and in positive amounts in both- extract and water. Plants mostly contain chemical compounds like saponins tannins, trypsin flavonoids, etc. as some secondary metabolites. Which are biologically active. (Lopez *et al.*, 2017). Phytochemical analysis of medicinal plants revealed the presence of phenolic compounds. These compounds are associated with antibacterial activities as well as curative properties against pathogens which are the same as our finding during the studies.

These results also demonstrated the existence of several phytochemicals, whose biological activity may have significant therapeutic value. The intensity of the colour on a scale of 4 (+, ++, +++, +++) indicates the outcome of the phytochemical analysis in *Zingiber officinale* extract.

Given that medicinal plants like *Zingiber officinale* are anticipated to be a valuable source for a variety of pharmaceuticals, it is no surprise that, according to the WHO, 80% of people in developed nations utilize traditional remedies made from plants. Investigating traditional medicinal plants is crucial to determine their qualities, safety, and effectiveness of therapy, as well as the best approach.

### DPPH radical scavenging capacity

Maximum inhibition activity in different solvents of Ginger rhizome extract was seen at 400 ug per ml. For methanol the reading was 98.602±0.26, water extract showed 96.148± 0.117 while ascorbic acid (standard) showed 99.472±0.026 %. The above study explains maximum DPPH radical scavenging capacity in decreasing order was ascorbic acid (standard), Methanol, and Water. So, it can be considered that methanol extract has better antioxidant potential than water (In the method of DPPH scavenging capacity).

TABLE III

### DPPH Radical Scavenging Capacity of Rhizome of *Zingiber officinale*

S/NO	Concentration of extract (µg/ml)	Inhibition (%)		
		Methanol extracts	Water extracts	Ascorbic acid (std)
1	100	85.135±0.093	86.331±0.117	95.880±0.142
2	200	89.795±0.046	91.141±0.188	97.741±0.261



3	300	92.498±0.026	95.589±0.163	98.157±0.142
4	400	98.602±0.026	96.148±0.117	99.472±0.026

**Note:** Values are given as Mean ± SD of three replicates

#### Antioxidant Potential (IC<sub>50</sub>)

The half-maximal inhibitory concentration (IC<sub>50</sub>) ranged from 6.220 ± 0.091 to 9.065 ± 0.128 (in the mean ± SD of three replicates).

TABLE IV

#### Antioxidant Potential (IC<sub>50</sub>) of Rhizome Extract of *Zingiber officinale*

S.No	Extracts/Standard	Methanol extracts	Water extracts
1	Ginger (rhizome)	9.065±0.128	6.220±0.091
2	Ascorbic acid (std)	1.131±0.091	1.131±0.091

**Note:** Values are given as Mean ± SD of three replicates

The concentration of the sample at which the inhibition percentage reaches 50% is IC<sub>50</sub>. The IC<sub>50</sub> values are shown in Table 4 above. The lower the IC<sub>50</sub> higher the antioxidant potential. Methanol extracts showed better antioxidant potential when compared with water extracts using the DPPH scavenging activity method. *Zingiber officinale* in water extract shows better antioxidant potential activity of 6.220±0.091 and 9.065±0.128 µg/ml in methanol extracts.

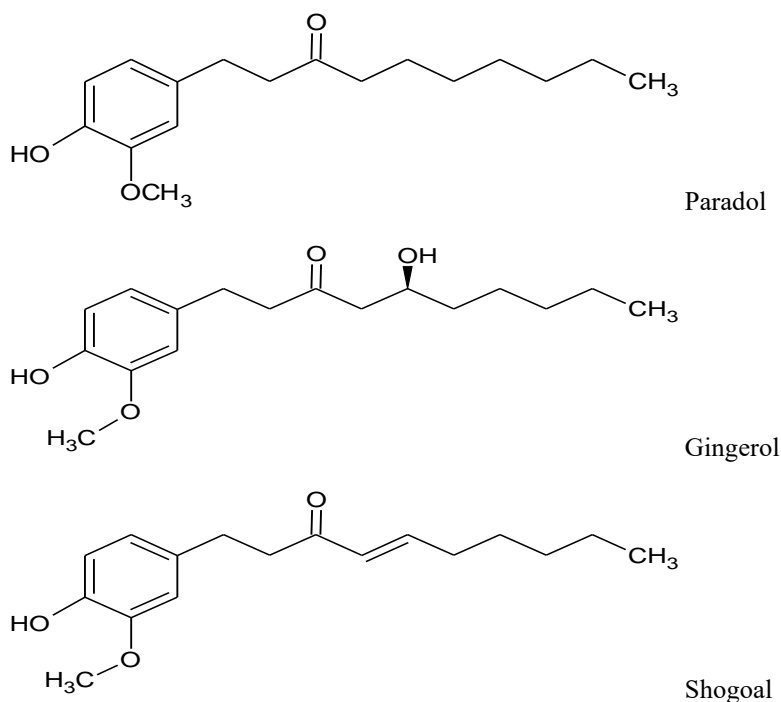
#### Antioxidant Activity

Polyphenol substances with high antioxidant activity, including 6-gingerol and its derivatives, are found in ginger root preparations. Antioxidant substances are frequently employed to prevent oxidative stress in cells and combat free radicals. Flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins, and isocatechins all contribute to antioxidant activity. The dried ginger rhizome's alcoholic extract has a total phenolic content of 870.1 mg/g extract. The extract has an IC<sub>50</sub> value of 0.64 µg/ml and 90.1% DPPH radical scavenging activity [22]. Ginger's antioxidant ability is a very important trait that can be employed as a preventative measure for a variety of ailments.

The key phytochemicals (Gingerols, shogaols, paradols, and zingerone) have been identified earlier studies also

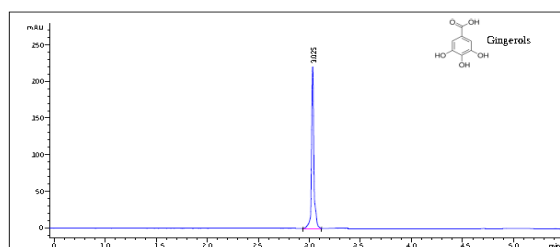
reveal the medicinal importance of ginger. Gingerols were shown to be the primary active ingredients in the fresh ginger rhizome [7]. Two different chemical groups, namely volatile oils, and non-volatile pungent molecules, are responsible for the sensory impression of ginger. Sesquiterpene hydrocarbons, primarily zingiberene (35%), curcumin (18%), and farnesene (10%), make up the majority of the volatile oil components in ginger [6]. Numerous components of these volatile oils are responsible for the characteristic flavour and scent of ginger. Gingerols, shogaols, paradols, and zingerone are non-volatile pungent substances that give off a "hot" flavour on the tongue. (Fig 1). The primary active ingredients in the fresh rhizome were discovered to be gingerols, a group of chemical homologs distinguished by the length of their unbranched alkyl chains. Additionally, the most pungent components in dried ginger are the shogaols, another homologous group, and the gingerols in their dehydrated state. Similar to gingerol, paradol is created when shogoal is hydrogenated. Oleoresins are moreover a component. Ginger includes carbs, waxes, lipids, vitamins, and minerals. Additionally, zingibain, a strong proteolytic enzyme, is found in the rhizomes of ginger [3-8].





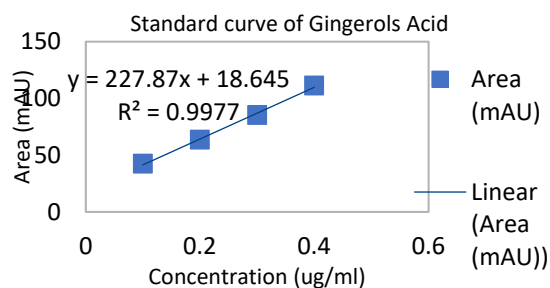
**Fig. 1:** Chemical components of ginger.

#### HPLC Chromatogram of Gingerols.



**Fig. 2:** HPLC Chromatogram of Gingerols

#### Gingerol Acid Standard Curve



In conventional Ayurvedic treatment, ginger is a crucial plant that is mainly used in different pathological

TABLE V

conditions, in particular for cough and asthma, including a mixture of fresh ginger juice, honey, and a small amount of fresh garlic juice. Additionally, 1-2 teaspoons of ginger juice mixed with honey are said to be an effective cough suppressant [5-10]. In addition to these, ginger is frequently used to treat a variety of ailments, including indigestion, tastelessness, loss of appetite, flatulence, intestinal, nausea, and vomiting as well as allergic reactions, acute and chronic cough, the common cold, fever, allergic rhinitis, sinusitis, acute chronic bronchitis, respiratory issues, pain, headache, and backache of any kind of swelled gum, painful tooth, and muscular catch [8-9-13].

#### Screening of Antibacterial Activities:

Minimum Inhibitory Concentration when the minimum concentration (mg/ml) of plant extract inhibits the growth of bacteria is called MIC (Minimum Inhibitory Concentration). It was determined that Ginger extract by the help of ELISA. For the study of MIC, plates have been incubated at 37°C for 16 hours. Further ELISA Test has been used to study the zone of inhibition.



Minimum Inhibitory Concentration (MIC) of Rhizome Extract

Rhizome extracts	<i>E. coli</i> (mg/ml)	<i>K. pneumoni a</i> (mg/ml)	<i>B. subtilis</i> (mg/ml)	<i>S. pneumoni a</i> (mg/ml)
<i>Z.offocinale</i>	642.6	730.7	55.03	2.442

The range of MIC was from 2.442 to 730.7 milligrams per ml. *Zingiber officinale* inhibit the growth of Bacteria-*S. pneumoniae* at 2.442, *B. subtilis* at 55.03, *E. coli* at 642.6, and *K. pneumoniae* at 730.7mg/ml. The antibacterial activity of rhizomes of ginger depends on the concentration of extract and the type of bacteria used in the experiment.

Simple Line Graph of Minimum Inhibitory Concentration

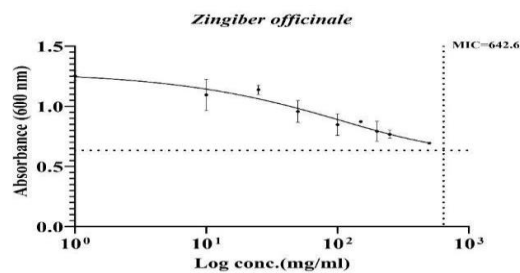


Fig: 3. A simple line graph to show the MIC of ginger for *Escherichia coli*.

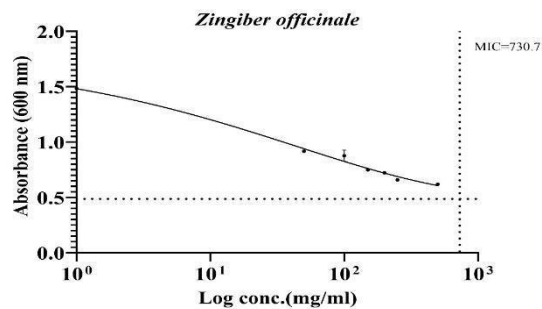


Fig: 4. A simple line graph to show the MIC of ginger for *Klebsiella pneumonia*

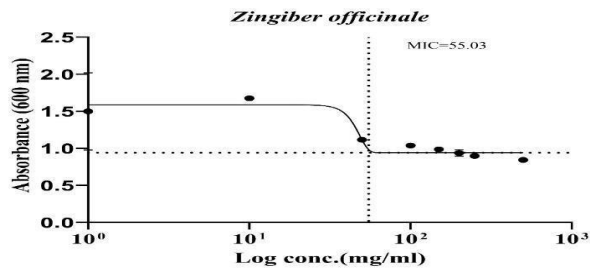


Fig: 5. A simple line graph to show the MIC of ginger for *Bacillus subtilis*

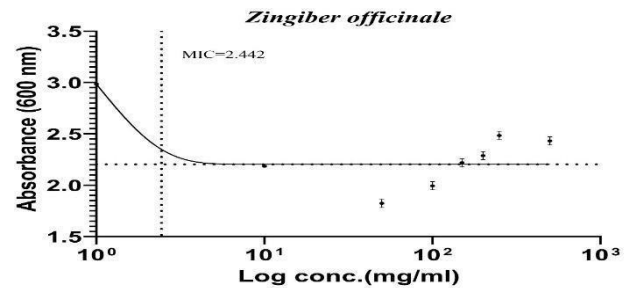
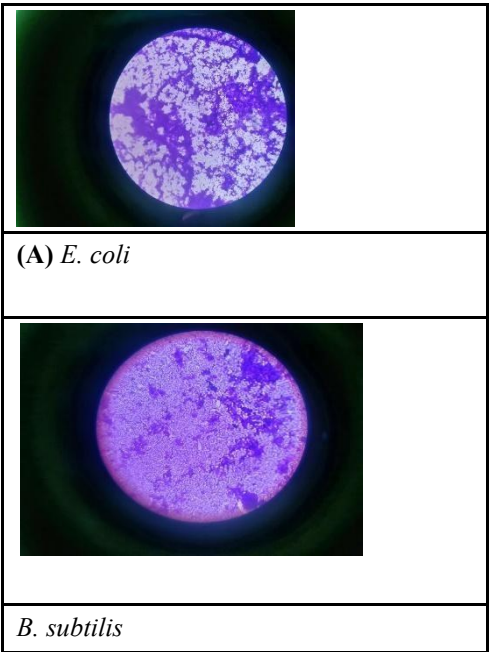


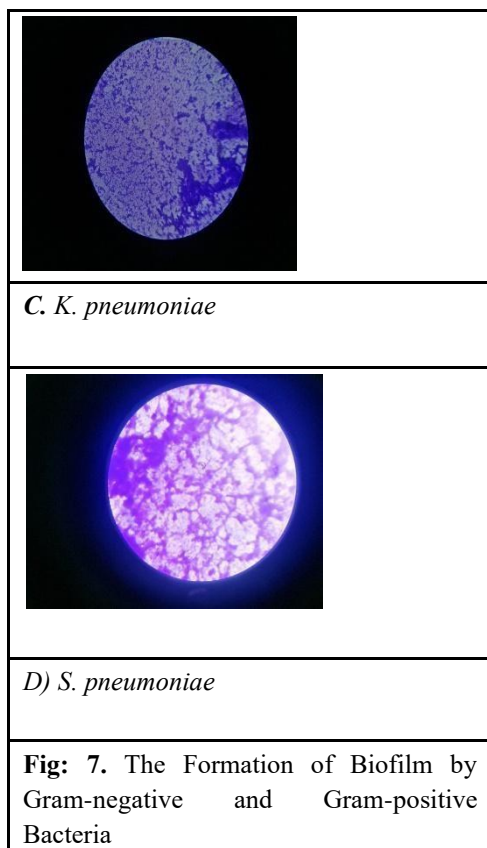
Fig: 6. A simple line graph to show the MIC of ginger for *Streptococcus pneumonia*

Bacteria Biofilm Formation

Bacteria adhere to the surface of a particular object and form a slim layer (smear) where it can reproduce. Form it on living and nonliving surfaces. Biofilm-forming bacteria show 5,000 times more antibiotic resistance capacity than nonbiofilm-forming bacteria. Biofilm has an extracellular matrix, interferes in the action of antibiotic action, and causes antibiotic resistance.







Bacteria fully in Regular font.

TABLE VI

Bacterial Biofilm Formation

S/No.	Bacterial Strains	Biofilm Formation
1	<i>Escherichia coli</i>	Biofilm formed
2	<i>Bacillus subtilis</i>	Biofilm formed
3	<i>Klebsiella pneumoniae</i>	Biofilm formed
4	<i>Streptococcus pneumoniae</i>	Biofilm formed

To reveal the established biofilm, a crystal violet assay was done for 10 minutes and was washed away properly with distilled water to remove the stain. With the help of an optical microscope, biofilm was observed. All the bacterial strains show good biofilm formation especially *E. coli* and *S. pneumoniae*, while *B. subtilis* and *K. pneumoniae* form less biofilm formation.

## Conclusion

Worldwide, medicinal plants have a significant economic impact. Traditional medicine uses the significant herb *Zingiber officinale* for its numerous therapeutic, ethnomedicinal, and nutritional benefits. Worldwide, ginger is used as flavouring and seasoning ingredient and is believed to offer a number of therapeutic benefits. Numerous compounds in ginger are responsible for its therapeutic benefits, including its anti-inflammatory, anti-microbial, anti-inflammatory, antioxidant, and anticancer effects. Some of its active ingredients, including the gingerols and shogoals, have been discovered to have anti-inflammatory, antioxidant, and anticancer activities. Numerous studies have shown that ginger has several health advantages and has the ability to treat a number of ailments, including nausea, vomiting, pain, and inflammation. To completely comprehend the impact and the processes of ginger on human health, additional study is necessary, particularly in clinical trials encompassing bigger and more diverse populations.

Additionally, although ginger is safe to eat in moderation, taking it as a supplement or in big doses might have negative effects and interfere with some drugs. On the basis of the above study, we conclude that Ginger works very effectively work on bacteria. Here, it works as an antibiotic because it controls the growth of bacteria. Antimicrobial activity shows variations due to different concentrations of methanol extract from ginger and different genera of bacteria i.e. *Escherichia coli*, *Streptococcus pneumoniae*, *Bacillus subtilis*, and *Klebsiella pneumoniae*. These bacteria can cause very harmful diseases in humans e.g. Pneumonia, different disorders of the lower abdomen, etc. Here, secondary metabolites are formed after bacteria-ginger infection. So, the above finding confirms the medicinal value and effectiveness of ginger against bacteria.

In summary, ginger is a prospective herbal cure with a variety of possible health advantages, and including it in a diet and lifestyle that are both well-balanced may help to improve general health and well-being.

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the above experimental work. All different strains of Bacteria were maintained at this lab.

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