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IN VITRO ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF GINGER EXTRACT

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Abstract

Introduction: Zingiber officinale, commonly known as ginger, is a popular spice and medicinal herb widely used in various traditional systems of medicine. The antioxidant activity of ginger extract has attracted considerable attention due to its potential in combating oxidative stress and associated health issues. Ginger extract has exerted its antibacterial effects through multiple mechanisms, such as disrupting bacterial cell membranes, inhibiting enzyme activity, and interfering with bacterial DNA replication. **Materials and Methods:** The anti-bacterial activity of ginger extract was analysed by the agar well diffusion method. First, the nutrient agar was uniformly spread in the Petri plates. The two bacterial strain Staphylococcus aureus and E.coli were used to examine the anti-microbial activity. After, 50, 100 µL of ginger extract was added to the Petri plates. The culture medium was incubated at 37°C for 24 h. Following 24 hours incubation, the zone of inhibition was measured. **Results:** Ginger extract shows great ABTS and DPPH radical savaging activity compared to standard ascorbic acid. The ginger extract sample shows the presence of many phytochemicals such as Alkaloids and Saponins, which aid in antioxidant property of the sample. It kills many microbial organisms like Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus). **Conclusion:** The findings of this study contribute to the growing body of evidence supporting the antioxidant potential of ginger extract. Further investigations could focus on elucidating the specific mechanisms by which ginger bioactive compounds exert their antioxidant effects, as well as evaluating the in vivo antioxidant activity in animal models and human subjects. The mechanisms underlying the antioxidant and antibacterial activities of ginger extract are likely multifaceted and involve the synergistic actions of its bioactive constituents

Keywords: Ginger Extract, Antioxidant, Antibacterial, Enzyme Activity.

INTRODUCTION

Ginger, often known as ginger root or ginger, is a flowering plant whose rhizome is used as a spice and a folk remedy. It is a herbaceous perennial that produces annual pseudostems, which are rolled leaf bases that are about one meter tall and have short leaf blades. Ginger, which has its roots in Southeast Asia but is most popular in India and China, has crossed cultural barriers to become a common component in dishes all around the world. With curries, stir-fries, marinades, gingerbread cookies, and beverages like ginger tea, it provides warmth and zing to both savory and sweet recipes. Directly emerging from the rhizome on distinct branches, the inflorescences contain flowers with pale yellow petals and purple margins. Several traditional medical systems use ginger as a popular spice and medicinal herb. For generations, people have treasured it for its unique flavor and scent as well as its conceivable health advantages (Fang D, Zhang C). A wide range of human health issues can benefit from ginger's therapeutic properties(Ambika, Manojkumar et al. 2019). It has been used for a long time to treat motion sickness, indigestion, and other digestive discomforts. It is a promising natural therapy for decreasing oxidative stress because of its anti-inflammatory and antioxidant qualities, which may reduce the chance of

developing chronic illnesses. Ginger's medicinal benefits are aided by a number of bioactive substances, such as gingerol, shogaol, paradols, and zingerone. An antioxidant study investigates and assesses the characteristics of chemicals known as antioxidants, which help shield cells from oxidative damage brought on by dangerous molecules known as free radicals(Senthil, Sundaram et al. 2022). Reactive oxygen species (ROS) production and the body's antioxidant defense mechanisms must balance each other out for oxidative stress to occur. Overproduction of ROS can cause cellular damage and play a role in the emergence of a number of illnesses, such as cancer, cardiovascular disorders, and neurodegenerative ailments. In a study on antioxidants, scientists look at the ability of various substances, which are frequently present in plants and food, to prevent or lessen the negative effects of free radicals. Vitamins (like vitamin C and E), minerals (like selenium), and phytochemicals (like flavonoids and polyphenols) found in fruits, vegetables, nuts, and other sources are some examples of these substances(Abdel-Maksoud EM, Daha AAEF).

The need for alternative antimicrobial drugs has been brought to light by the rise of bacteria that are resistant to antibiotics. Both gram-positive and gram-negative types of bacteria have been successfully eradicated by ginger extract. It works in a variety of ways to kill bacteria, including by interfering with bacterial DNA replication, rupturing bacterial cell membranes, and blocking enzyme activity. A thorough understanding of ginger extract's potential as a substitute antibacterial agent will result from investigating the variables that affect its potency, including extraction techniques, concentration, and interactions with bacterial cell structures.Understanding ginger extract's antibacterial properties has significance for creating new therapeutic approaches as well as expanding our understanding of natural substances as prospective treatments for the antibiotic resistance dilemma.(Thagfan FA, Dkhil MA) Exploring the potential of ginger extract as a natural antibacterial agent has promise for both conventional medicine and contemporary healthcare practices as we continue to face issues brought on by drug-resistant bacteria. Due to its ability to battle oxidative stress and the health problems it is connected with, the antioxidant activity of ginger extract has received a lot of attention(Prathap and Jayaraman 2022). Supplemental ginger has been associated with elevated levels of antioxidant enzymes and decreased oxidative indicators in both animal and human studies. Ginger is thought to provide anti-inflammatory effects, better cardiovascular health, and maybe anticancer characteristics. These advantages are thought to be influenced by its antioxidant properties. Understanding the antibacterial activity of ginger extract has implications not only for developing novel therapeutic interventions but also for advancing our knowledge of natural compounds as potential solutions to the antibiotic resistance crisis (). As we continue to face challenges posed by drug-resistant bacteria, exploring the potential of ginger extract as a natural antibacterial agent holds promise for both traditional medicine and modern healthcare practices (Ponmanickam, Gowsalya et al. 2022).

MATERIALS AND METHODS

Freshly harvested ginger rhizomes (500 g) each were acquired from a local food market in Chennai, packed in airtight bags, and transported to Saveetha Dental College and a hospital food biochemistry laboratory in India. Samples were carefully cleaned under tap water, drained to eliminate excess water, and grated for extraction in the laboratory

Plant Collectıon

Fresh *ginger rhizomes (2)*were rinsed with distilled water, and dried in the shadow. The *ginger rhizomes* powder 5g was placed in a cellulose thimble with medium porosity (10–15 m) and then continuously extracted using the Soxhlet method with 100 mL of 95% ethanol for 12 hours at 70 °C. When the recirculation of ethanol became obvious after 12 hours, the 100% yield of the extraction was guaranteed. A rotary evaporator was used to condense the resulting green extract to dry residue, which was then refrigerated until use.

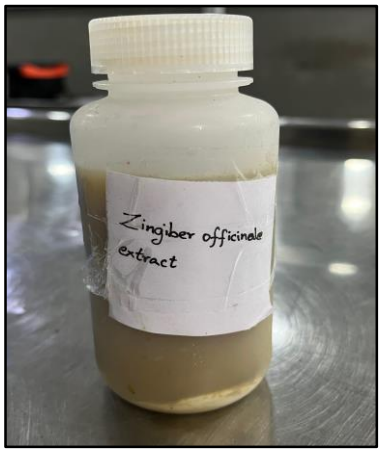


Fig 1: Concentrated *Zingiber officinale* extract

Source: Saveetha Dental college

DPPH Analysis

The samples were kept at 30°C and incubated for 15 minutes in the dark. A spectrophotometer was used to quantify the decrease in absorbance at 517 nm in comparison to ethanol. A blank sample containing the same quantity of ethanol and DPPH was generated and tested every day after the spectrophotometer was zeroed with ethanol.() Daily fresh preparations of the DPPH solution were made, and it was kept at 4°C in the dark and covered with aluminum foil. After 15 minutes, the radical scavenging activities of the tested samples were calculated. 50 to 250 µg/ml samples were taken, and ascorbic acid was used as standard one.

ABTS Assay

Using the ABTS radical cation decolorization assay , which is based on the reduction of ABTS+• radicals by the antioxidants of the analyzed plant extracts, the ability of plant extracts to scavenge free radicals was also investigated. In deionized water, ABTS was dissolved to a 7 mM concentration. By combining ABTS solution with 2.45 mM potassium persulfate (final concentration) and letting the combination sit undisturbed at room temperature for 12 to 16 hours before use, ABTS radical cation (ABTS+*) was created. The ABTS+ the solution was diluted for the investigation to an absorbance of 0.7 at 734 nm in deionized water or ethanol.A suitable solvent blank reading (Am) was obtained. The absorbance reading was collected 10 minutes after the first mixing of 3 mL of ABTS+• solution with 100 uL of aqueous or ethanolic (depending on solubility) plant extract solutions (Ag). On the day of preparation, all solutions were applied, and each determination was made three times(Prathap and Jayaraman 2022).

Antimicrobial Activity

To assess the antimicrobial activity of plant or microbial extracts, the agar well diffusion method is frequently utilized [32], [33]. The agar plate surface is inoculated using a process similar to the disk-diffusion approach in which a volume of the microbial inoculum is dispersed across the entire agar surface. Next, a volume (20-100 L) of the antimicrobial agent or extract solution is put into the well by aseptically drilling a hole with a diameter of 6 to 8 mm using a sterile cork borer or tip. (7)The test microorganism is then placed on an appropriate agar plate, and the incubation process is continued. The antibiotic ingredient spreads across the agar media and stops the tested microbial strain from growing.

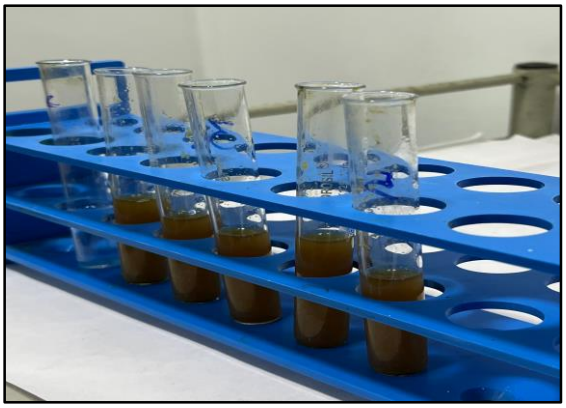


Fig 2: *Zingiber officinale* extract solution is taken in a sterile test tube, antimicrobial agent ranging from volumes 20ml-100ml is added

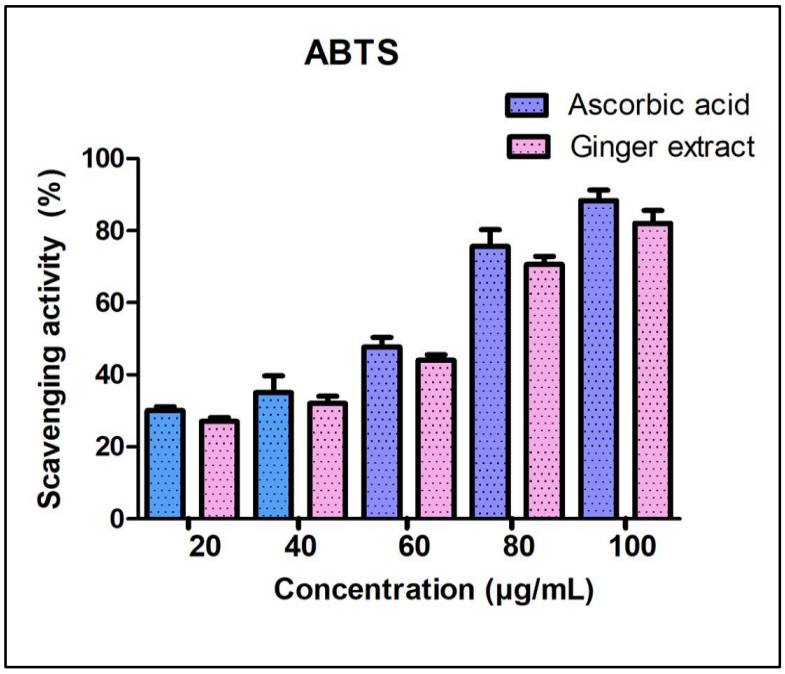
Source: Saveetha Dental college

Cytotoxicity

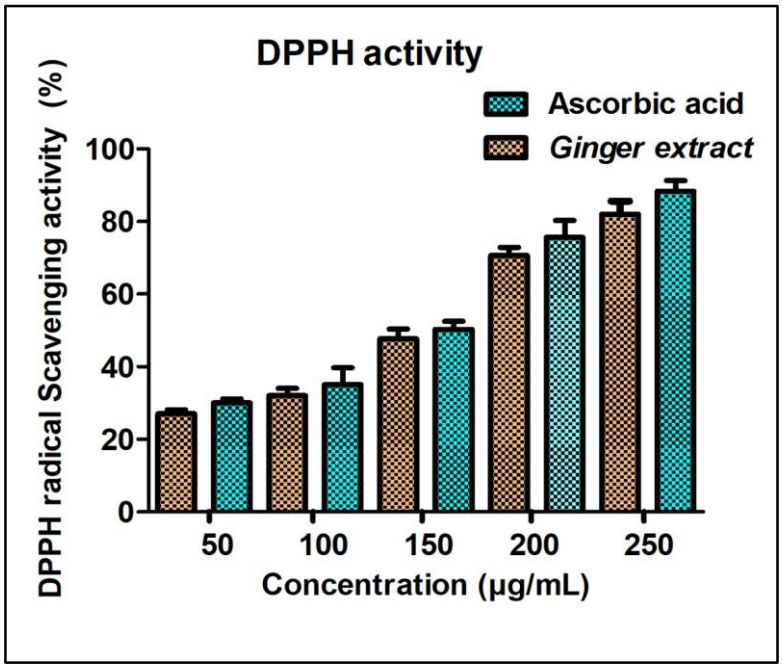
Add 5 mg/ml of MTT in Dulbecco's Phosphate saline (DPBS), which has a pH of 7.4.Add MTT solution in a sterile, light-protected container after filter-sterilizing it with a 0.2 M filter. MTT solution should be kept in a dark, protected area at -20°C or 4°C for long-term storage. Solution for Solubilization: Work in a ventilated fume hood and select an appropriate solvent-resistant container.Dimethylformamide (DMF) 40% (vol/vol) in glacial acetic acid 2% (vol/vol) are prepared. SDS sodium dodecyl sulfate, 16% (wt/vol), is added and dissolved. Set the pH to 4.7.Store at room temperature to prevent SDS from precipitating. In order to dissolve SDS, warm the solution to 37 °C and mix.Prepare test chemicals and cells in 96-well plates with a final volume of 100 l/well.For the necessary amount of exposure, incubate.To reach a final concentration of 0.45 mg/ml, add 10 l of MTT Solution per well.At 37 °C, incubate for 1 to 4 hours. For each well, add 100 l of the Solubilization solution to dissolve the formazan crystals. Mix thoroughly to ensure full solubilization. The absorbance was recorded at 570 nm.

RESULTS

Ascorbic acid is a basic low-molecular antioxidant that plays a role in the regulation of the levels of reactive oxygen species (ROS) and may be used to assess the efficacy of other antioxidants, making it a standard oxidative measure. Similar to this, plant polyphenols that have antioxidant potential are less reactive and require more time to establish a stable reaction.



Graph 1: standard antioxidant Ascorbic acid as it regulates the level of ROS as early as the stage of their formation. The methanolic extract of ginger showed significant nitric acid scavenging activity in a dose-dependent manner. The inhibition concentration (IC 50) was calculated to be 80µg/ml

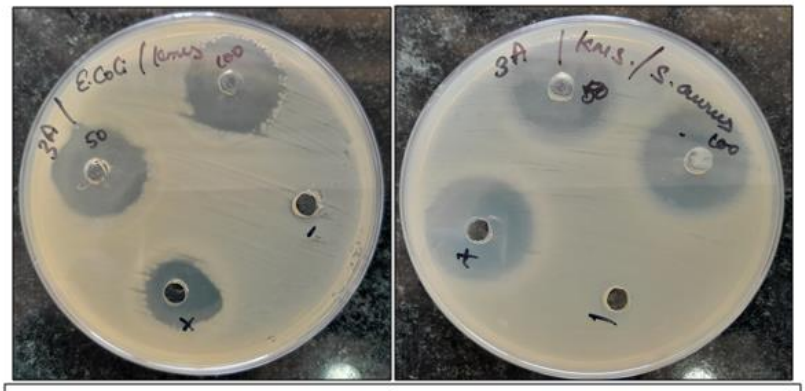


Graph 2: Mean (+ DPPH activity) ginger extract is compared to standard ascorbic acid as it responds more favorably and reacts urably and reacts much more quickly, a steady absorbance value appears after only a short period of time. 50 100 150 200 250 is taken from minimum to maximum dose. The inhibition concentration was calculated to be 150µg/ml.

Phytochemical Analysis

Table 1: The phytochemical analysis of ginger extract shows the presence of the essential alkaloids, phenolic compounds, saponins, oil and fat, protein and amino acids, flavonoids and carbohydrates in optimal quantities

S.No.	Phytocompounds	Ginger Extract
1	Alkaloids	Present
2	Phenolic compounds	Present
3	Saponins	Present
4	Oil and Fat	Absent
5	Protein & Amino acid	Absent
6	Flavonoids	Present
7	Carbohydrates	Present



S.No	Micro organisms	Zone of Inhibition			
		Positive Control	Negative Control	50 µL	100 µL
1	*E.coli*	5 mm	-	11 mm	17 mm
2	*S.aureus*	7 mm	-	14 mm	19 mm

Figure 3: Antimicrobial Activity Of Ginger Extract

Antimicrobial resistance (AMR) is one of the major issue all around the world . The microbial species E.coli and S.aureus were taken for the evaluation minimum inhibition concentration (MIC) is the lowest concentration of plant extract will inhibit the growth of the microorganisms .The zone of inhibition for E.coli 5mm, 11mm, 17mm, (50,100,150µL control, respectively). The zone of inhibition of S.aureus 7mm, 14mm, 19mm (100,150µL control respectively).

DISCUSSION

Studies on the in vitro antioxidant activity of ginger extract have provided information on its potential advantages for human health. The extract's high concentrations of polyphenols, flavonoids, and anthocyanins - all of which are well-known for having high antioxidant capacities—have contributed to its appeal as a potential natural source of antioxidants.(8)

Researchers have used a number of in vitro experiments, including as the DPPH, ABTS, and FRAP assays, to demonstrate the extract's ability to successfully scavenge free radicals and neutralize oxidative species(9). Bioactive substances including gingerol, shogaol, paradols, and zingerone, which are recognized for their antioxidant qualities, are present in ginger extract. In order to combat free radicals, unstable chemicals that might harm cells, antioxidants are essential. The capability of ginger extract to reduce oxidative stress is influenced by its capacity to scavenge free radicals(10) .Numerous chronic illnesses, such as cardiovascular ailments, cancer and many neurological diseases are thought to be influenced by oxidative stress. Many studies have proven that ginger extracts's ability to to reduce the oxidative damage to the cell, likely to be highlighting the disease-prevention or disease-mitigation potential of this substance. Ginger extract has been known to strengthen the body's natural antioxidant defense mechanism in addition to scavenging free radical substances. This can be accomplished by increasing antioxidant rich enzymes in the body like catalase and superoxide dismutase, which helps in strengthening the body's defence attack against oxidative stress(11) .The anti-inflammatory and antioxidant activities of ginger extract are strongly related to one another. Numerous illnesses are fueled by chronic inflammation, and ginger's capacity to do so is part of what makes it so healthy overall

Both gram-positive and gram-negative bacteria in many cases, have been shown to be sensitive to ginger extract's antibacterial properties. The presence of bioactive substances that prevent bacterial growth and replication is thought to be the cause of this broad-spectrum action(12) .Also, several processes are known to help ginger extract to exert its antimicrobial properties in the medical field. It has the ability to damage bacterial cell membranes, causing cellular contents to flow out which ultimately leads to bacterial death. Further preventing bacterial growth, ginger extract can also obstruct DNA replication and bacterial enzyme function. The rise of many pathogens that are resistant to many third-generation antibiotics are a problem for world health. The antioxidant and antibacterial properties of ginger extracts proves to be a potential method for overcoming the resistance against such disease-causing pathogens(13) .Ginger's multiple modes of action make it less probable for bacteria to acquire resistance than antibiotics that focus on particular routes.

FUTURE SCOPE

Ginger extract promises versatile benefits in the medical and health industry due to the natural antioxidant and antibacterial benefits of the herb. It aids in fighting oxidative stress-related disorders. Ginger extract can also be used in dietary and health treatment plans. This might involve creating functional meals or supplements based on ginger that improve general health. The antimicrobial properties of ginger extract may have uses in complementary and alternative medicine. It can be used as a preventative step against bacterial infections or in combination with other antibiotics to boost their efficacy. To identify and describe the precise bioactive substances in ginger that are responsible for its antioxidant and antibacterial properties, more study is required. This could result in the creation of medicinal products based on derivatives of ginger. However, clinical studies are required to confirm the effectiveness of ginger extract in people. The doses, possible adverse effects, and long-term consequences on health should all be investigated in upcoming research.

CONCLUSION

The research found that phenolic components are abundant in ginger extract. Local ginger outperformed hybrid ginger in terms of total phenolic, flavonoid, and vitamin C content in ginger extracts. Water was not as effective as organic solvents in extracting phytochemicals, with ethanol being more effective than acetone and methanol. However, ethanol, methanol, and acetone were less effective than water at extracting vitamin C. The amount of total phenolic and flavonoid content was positively linked with the antioxidant activity of extracts measured using DPPH tests. Local ginger extracts in ethanol, acetone, and methanol demonstrated excellent antioxidant activity. Based on effectiveness and safety, the study suggests extracting phytochemicals using ethanol. It is also advised to calculate the specific phenolic acids and flavonoids in ginger extracts in order to promote their raw use of antioxidants and antibiotics.

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Conflict of Interest

None to declare.

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