



RESEARCH ARTICLE

ZINGIBER OFFICINALE: PHYTOCHEMICAL ANALYSIS AND EVALUATION OF ANTIMICROBIAL ACTIVITY IN COMBINATION WITH COMMERCIAL ANTIBIOTICS

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ABSTRACT

Zingiber Officinale is used in the regular diet in many Asian countries. Chemical analysis of ginger shows that it consists of more than 400 different compounds. In the present study, antimicrobial activity of aqueous, ethanol and acetone extracts of *Zingiber Officinale* were evaluated against *S. aureus* (ATCC 25923) and *E. coli* (ATCC25922). Ethanol extract demonstrated a higher antibacterial activity than the acetone and aqueous extract. These extracts were prepared from fresh *Zingiber* rhizomes. These extracts were evaluated for their part in increasing antibacterial activity of streptomycin and tetracycline against *S. aureus* (ATCC 25923) and *E. coli* (ATCC25922). The antibacterial activity of streptomycin and tetracycline were enhanced against the test organism in the presence of these extracts. Phytochemical analysis gave positive results for steroids, triterpenoids, glycosides, phenolic compounds, flavonoids, tannins, saponins, carbohydrates, amino acids and proteins. *Zingiber Officinale* contains pharmacologically bioactive constituents that may be responsible for its activity against test organisms.

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INTRODUCTION

Medicinal plants are cheap and renewable sources of pharmacologically active substances and are known to produce certain chemicals that are naturally toxic to bacteria (Basile et al, 1999). The rhizome (underground stem) of *Zingiber Officinale* is used as a spice and also as a medicine. It can be used fresh, dried and powdered, or as a juice or oil. *Zingiber officinale* has been used as a medicinal plant in Asia, India, Jamaica and Nigeria. In China, ginger has been used to aid digestion, treat stomach upset, diarrhea and nausea for 2000 years (Azu and Onyeagba, 2007). Ginger has a wide range of action on the human body and has been found effective in the treatment of cataract, heart disease, migraines, struck amenorrhea, athlete's foot, bursitis, chronic fatigue, cold, flu, coughs, depression, dizziness, fever, erectile difficulties, kidney stones, renal disease and viral infection. It is a valued remedy for coughs and bronchitis and also serves as a soporific in fever its natural diuretic stimulates the kidney to flush out toxins faster. The rhizomes of *Zingiber officinale* contain substances with several properties of interest, including bactericidal, fungicidal, antiviral, antiulcerative and antioxidant activity; they also contain enzymes with proteolytic activity (Millar, 1998; Kim et al., 2008; Ali et al., 2008; Takara et al., 2005). These enzymes are called zingibain

and exhibit collagenase activities (Choi and Laursen, 2000; Shukla and Singh, 2007). Their activities are similar to those of the protease papain, which is associated with the ripening of fruit and tenderizing of meat. These characteristics make the enzyme a promising alternative for culinary and industrial applications (Kim and Lee, 1995). India is one of the country that extensively use herbal medicines to meet their healthcare needs. Here, the herbal drug market is around 1 billion U.S dollars, and the export of plant-based crude drugs is around 80 million U.S dollars. However, unlike China, India has not been able to capitalize on this herbal wealth by promoting its use in the developed world despite their renewed interest in herbal medicine (Kamboj, 2000). Modern scientific research has revealed numerous therapeutic properties of ginger including antioxidant effects, ability to inhibit the formation of inflammatory compounds and direct anti-inflammatory effects (George Mateljan Foundation, 2014). Ginger can be available in different commercial products like cookies, candy, teas, tinctures, sodas, jam, beer, capsule and syrup (Maxwell, 2008). The chief active constituents of ginger are Volatile oil (zingiberene, zingiberol, D-camphor), Shogaols, Diarylheptanoids, Gingerols, Paradol, Zerumbone, 1-Dehydro-(10) gingerdione, Terpenoids and Ginger flavonoids (Baliga et al., 2012). Shogaols and Gingerols are responsible for ginger's pungency (Suekawa et al., 1984). Ginger has wide range of biological activities that are attributed to its active constituents (Shukla and Singh, 2007). In current study, antibacterial

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activity of the ethanol, acetone and aqueous extract were assessed separately and in combination with streptomycin and tetracycline against pathogenic bacteria.

MATERIALS AND METHODS

Sample collection

The rhizomes of Ginger were collected from market of Jalna, (Maharashtra, India). The samples were washed thrice using tap water followed by distilled water and were dried under shade in hygiene conditions for 10-12 days. All the materials were ground in an electric grinder to produce fine powder. Powdered material was stored at 4°C in an air tight bottle.

Preparation of leaf extracts

For aqueous extract 10gm of powdered material added to 100ml of distilled water and then kept overnight. This was filtered through Whatman's filter paper and filtrate was used as aqueous extract. Same procedure is repeated for Ethanol and acetone extract. These extracts were stored at 4°C for further use.

Test organism

Bacterial cultures were selected from American type culture collection (ATCC). The strain used for the study were *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). These were grown on their respective selective media and purity was determined by morphological and biochemical characterization.

Inoculum preparation

Loopful of pure culture from selective media was picked up and inoculated in Muller Hinton Broth (Himedia). It was incubated at 37°C for 3-7 hrs. Until moderate turbidity develops. Inoculum turbidity was compared with that of 0.5 McFarland standard.

Preparation of Disc

Whatman's filter paper no.1 was punched to get disc of 6mm diameter. These discs were sterilized under UV light. Each sterile disc was impregnated with ethanol extract, acetone extract, aqueous extract and excess of solvent was dried in controlled temperature.

Antimicrobial activity of extract

The antimicrobial activity of the extract was evaluated by standard disc diffusion method (Baur *et al.*, 2012). Plates of Muller Hinton agar (Himedia) medium having media up to 4 mm were prepared. After solidification lawn of inoculum was prepared on to agar plates for each organism. Inoculum was taken by soaking the sterile swab (Himedia) in prepared inoculum of test organism i.e. *Staphylococcus aureus* and *Escherichia coli* and spread over the agar plates for respective organism. Ethanol extract disc, acetone extract disc and aqueous extract disc of *Ginger* was applied and incubated at 28-30°C for 16-18 hours.

Disc diffusion assay to evaluate combined effects

Disc diffusion method was used to evaluate in vitro antibacterial activity of streptomycin against *Staphylococcus*

aureus and *Escherichia coli* on Muller Hinton Agar (Himedia). The standard streptomycin and Tetracycline disc (10mcg, Himedia). To determine combined effect, each standard paper disc was further impregnated with 20µl of each single extract. Muller Hinton Agar plates were inoculated with *Staphylococcus aureus* and *Escherichia coli*. Standard antibacterial streptomycin disc and Tetracycline were used as positive control and streptomycin disc and Tetracycline impregnated with aqueous, ethanol, and acetone extract were placed onto Muller Hinton Agar plate inoculated with test organisms. Aqueous, Ethanol, and Acetone extract discs were used as a negative control. These plates were incubated 16-18 hours. After incubation, the zones of inhibition were measured. The assays were performed in triplicate.

Assessment of increase in fold area

The increase in fold area was assessed by calculating the mean surface area of the inhibition zone of each antibacterial agent (streptomycin and Tetracycline) and antibacterial agent plus extract. The fold increase area of different test organism for antibacterial agent and for antibacterial agent plus extract was calculated by equation $(B^2 - A^2)/A^2$, where A and B were zones of inhibition for antibacterial agent and antibacterial agent + extract, respectively.

Phytochemical analysis

Phytochemical tests were done to find the presence of the active chemical constituents such as alkaloid, flavonoids, glycosides, triterpenoids, steroids, tannin and phenols, reducing sugar, carbohydrates and protein and amino acids by the following procedure. (Kokate, 2000, Harbone, 1999; Tiwari *et al.*, 2011)

Tests for Alkaloids

To the extract, dilute hydrochloric acid was added, shaken well and filtered. With the filtrate, the following tests were performed.

Mayer's reagent test

To 3 ml of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of creamy precipitate indicates the presence of alkaloids.

Tests for Carbohydrates

Molisch test

2 ml of aqueous extract was treated with 2 drops of alcoholic α -naphthol solution in a test tube and then 1 ml of concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrates.

Tests for Reducing Sugars

Benedict's test

Equal volume of Benedict's reagent and extract were mixed in a test tube and heated on a water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount

of reducing sugar present in the test solution which indicates the presence of reducing sugar.

Tests for Flavonoids

Alkaline reagent test

The extract was treated with few drops of sodium hydroxide solution separately in a test tube. Formation of intense yellow color, which becomes colorless on addition of few drops of dilute acid indicates the presence of flavonoids.

Tests for Glycosides

Borntrager's test

To 3 ml of test solution, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene or chloroform was added and it was shaken well. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red color in ammonical layer indicates the presence of anthraquinone glycosides.

Tests for Tannin and Phenolic compounds

Ferric chloride test

A small amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds.

Test for Saponin

Froth test

The extract was diluted with distilled water and shaken in a graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

Tests for Protein and Amino acids

Ninhydrin test

3 ml of the test solution was heated with 3 drops of 5% Ninhydrin solution on a water bath for 10 minutes. Formation of blue color indicates the presence of amino acids.

Tests for Triterpenoids and Steroids

Salkowski's test

The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layer turns red, sterol is present. Presence of golden yellow layer at the bottom indicates the presence of triterpenes.

RESULTS AND DISCUSSION

The antibacterial activity of Acetone, Ethanol and aqueous extract of Ginger along with Streptomycin and Tetracyclin against *E.coli* and *S. aureus* were shown in Table 1. Ethanol extract shows highest 17mm zone against *E.coli* and 13 mm

Table 1. Antibacterial activity of Ethanol, Acetone and Aqueous extract of Ginger

Test Organism	Zone of inhibition of <i>Ginger</i> Extract in mm			Zone of inhibition of Streptomycin in mm	Zone of inhibition of Tetracyclin in mm
	Ethanol	Acetone	Aqueous		
<i>S. aureus</i>	13	15	12	23	18
<i>E. coli</i>	17	17	09	24	20

Table 2. Zone of inhibition of Streptomycin against test organism in absence and in presence of *Ginger* extract at content 20µl per disc

Test Organism	<i>Ginger</i> Extract	Streptomycin +Extract (B)	Streptomycin (A)	Increase in Fold B^2-A^2/A^2 Area
<i>S. aureus</i>	Ethanol	25mm	23mm	0.18
	Acetone	28mm	23mm	0.48
	Aqueous	23mm	23mm	00
<i>E. coli</i>	Ethanol	28mm	24mm	0.36
	Acetone	26mm	24mm	0.17
	Aqueous	28mm	24mm	0.36

Table 3. Zone of inhibition of Tetracyclin against test organism in absence and in presence of *Ginger* extract at content 20µl per disc

Test Organism	<i>Ginger</i> Extract	Tetracyclin +Extract (B)	Tetracyclin (A)	Increase in Fold B^2-A^2/A^2 Area
<i>S. aureus</i>	Ethanol	22mm	18mm	0.49
	Acetone	18mm	18mm	00
	Aqueous	20mm	18mm	0.23
<i>E. coli</i>	Ethanol	24mm	20mm	0.44
	Acetone	25mm	20mm	0.56
	Aqueous	22mm	20mm	0.21

Table 4. Phytochemical analysis of plant extracts

Phytochemical test	Ethanol extract	Acetone extract	Aqueous extract
Tests for Alkaloids	-	-	-
Tests for Carbohydrates	+	-	+
Tests for Reducing Sugars	+	-	+
Tests for Flavonoids	+	+	+
Tests for Glycosides	+	-	+
Tests for Tannin and Phenolic compounds	-	+	+
Test for Saponins	+	-	+
Tests for Protein and Amino acids	-	+	-
Tests for Triterpenoids and Steroids	+	-	+

(+) indicates presence while (-) indicates the absence of the components

zone against *S. aureus*, followed by acetone extract shows 17mm against *E. coli* and 15 mm zone against *S. aureus*, whereas aqueous extract shows 9mm zone diameter against *E. coli* and 12 mm zone against *S. aureus*. Standard antibiotic Tetracyclin shows 24 mm zone diameter against *E. coli* and 23 mm zone against *S. aureus*. Standard antibiotic Tetracyclin shows 20 mm zone diameter against *E. coli* and 18 mm zone against *S. aureus*. In the in vitro antibacterial activity of Streptomycin and Tetracyclin, an antibacterial agent that is widely used against Bacterial infection, was used as positive control for comparison with *Ginger* extracts. The diameter of zone of inhibition and increase in fold area for all the test organism was measured. The antibacterial activity of Streptomycin and Tetracyclin increased significantly in presence of ethanol, acetone and aqueous extract of *Ginger* shown in Table 2 and 3.

Phytochemical analysis

The phytochemical analysis of plant extracts using acetone, ethanol and aqueous was showed in Table 4. Ethanol extract of *Ginger* showed the presence of glycosides, flavonoids, saponins, carbohydrates, reducing sugar, triterpenoids and steroids. Amino acids and proteins, flavonoids, tannin and phenolic compound were observed in acetone extract of *Ginger* but there was absence of carbohydrates, reducing sugar, triterpenoids and steroids. Glycosides, flavonoids, saponins, tannin and phenolic compound, reducing sugar, carbohydrates, triterpenoids and steroids were found in presence of aqueous extract of *Ginger*. Ethanol, Acetone and Aqueous extract of *Ginger* shows absence of alkaloids. In particular the flavonoids were reported to be responsible for ethno medicinal plants (Singh and Bhat, 2003). Water was used as a solvent to extract the *Zingiber officinale* juice. Imokawa (2008) indicated that the substances responsible for the spicy characteristics of *Z. officinale* are, for the most part, insoluble in water, whereas the desired proteases are extracted with water. Therefore, the aqueous extract is non-irritating and safe to use on human skin (Thompson, 1973). The results in the antimicrobial assay corroborate Jagetia et al. (2003), who administered *Zingiber officinale* intraperitoneally and observed antimicrobial activity against *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli* and *Candida albicans*.

Conclusion

Zingiber officinale marked inhibitory effect on *S. aureus* and *E. coli* with ethanolic, methanolic and Aqueous extracts, The results indicated that the plant have growth inhibitory effect in vitro against pathogenic bacteria. Phytochemical constituents such as steroids, alkaloids, flavonoids, tannins, phenol and several other aromatic compounds are secondary metabolites of plants that serve a defence mechanism against predation by many microorganisms, insects and herbivores. These secondary metabolites exert antimicrobial activity through different mechanisms. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea, colitis and dysentery etc. The alkaloids contain in plants are used in medicine as anesthetic agents. Ginger rhizome extract and their components can be used as alternative and effective novel therapeutic strategy. The combined effects of a standard antibacterial agent (Streptomycin and Tetracyclin) with extracts against *S. aureus* and *E. coli* similarly a new finding. Further, it can be concluded that extract alone or their

formulations (combination) can be used as effective agents against human bacterial pathogen.

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