

**Antibacterial, anti-oxidant and *in vitro* anticancer analysis of *Zingiber officinale* (L.) Rosc.**

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**Abstract**

Ginger is considered as an important spice with many clinical potential activities. The present study was undertaken to determine antimicrobial, antioxidant and *in vitro* anticancer activities of rhizome extract of *Zingiber officinale*. Antimicrobial activity was tested against *Staphylococcus aureus*, *Bacillus subtilis*, *Vibrio cholera*, *Vibrio parahaemolyticus*, *Pseudomonas aeruginosa*, *Enterotoxigenic Escherichia coli* (ETEC) and *Salmonella typhi* by disc diffusion assay. Antioxidant activity was determined by DPPH free radical scavenging assay and ABTS free radical scavenging assay. The ethanolic extract showed more activity against *Vibrio cholera*, *Vibrio parahaemolyticus*, ETEC and *Salmonella typhi* at 5–25 mg/well. The extract also exhibited dose dependent scavenging of DPPH and ABTS radicals. Scavenging of DPPH radicals was more efficient than that of ABTS radicals as revealed by the low IC<sub>50</sub> value. MCF-7 breast cancer cells were used to investigate the *in vitro* anticancer activity of ethanol extracts of ginger. The inhibitory activity of the extracts against MCF-7 was carried out using colorimetric tetrazolium salt (MTT) assay. The results showed considerable anticancer activity in MCF-7 cancer cells, with an IC<sub>50</sub> value of 10 µg/ml. Ginger extract effectively inhibited proliferation of MCF-7 cells in a dose-dependent manner over 24h. Thus the compounds of ginger displayed potential for antioxidant, antibacterial and anticancer agents.

**Key words:** *Zingiber officinale*, antibacterial, anti-oxidant, MCF-7 and *in vitro* anticancer.

## 1. Introduction

In traditional medicine system plants have been used as a source of therapeutic agents, due to its bioactive content (Pieters and Vlietinck 2005; Balunas and Kinghorn 2005). Particularly, fruits, vegetables and spices contains high health promoting components such as vitamins, minerals, antioxidants and prebiotics (Omenn et al., 1996). So, the interest in usage of vegetables and spices for medicinal purpose was renewed. Spices are fruit, roots, seeds, bark or berry parts of the plant which were used in foods to add flavours, colour and also to preserve them (Abbas and Halkman 2004). These plant species have constituents which possess biological activity more specifically antibacterial, antifungal, antioxidant and anticancer properties. Extensive research has been carried out in traditional medicine in different plant species to explore their therapeutic potentials (Policegoudra et al., 2007). These experimental results show that they exhibit excellent antioxidant activity which involves eradicating the free radicals, reactive oxygen and nitrogen species their by controlling the emergence of disease and antibacterial property against several pathogenic bacterium which are resistant to many antibiotics causing mortality and morbidity to people community, they have also proved to exhibit anticancer qualities. Therefore the exploration and utilization of natural substances from plants are more commendably desired.

The rhizomes of *Zingiber officinale*, belonging to the family Zingiberaceae is the most widely used spices. It is a perennial rhizomatous monocot. Traditionally, ginger is used for the treatment against various diseases like stroke, asthma, diabetes, nervous disorder (Thomson et al., 2002). Ginger has also exhibited excellent activity against rheumatoid arthritis and osteoarthritis as they act as a natural pain killer against inflammation.

The purpose of this study was to analyse the effectiveness of ginger rhizome ethanolic extract towards its potency as an antibacterial agent against standard and antibiotic resistant clinical isolates plus its anti-oxidant and *in vitro* anticancer abilities.

## **2. Material and methods**

### **2.1. Plant Material**

Healthy *Zingiber officinale* rhizomes were collected from the Gandhi market, Tiruchirappalli, Tamil Nadu, India, during August 2015. The rhizomes were washed thoroughly with a few drops of Tepol in running tap water to remove surface debris and then rinsed with distilled water. Further, they were sliced and dried in shade for 2-4 days. The dried rhizomes were ground into fine powder using a mixer grinder.

### **2.2.Extraction procedure**

Ten grams of *Zingiber officinale* rhizome powder was extracted with 200 ml of 99.5% ethanol (Fisher Scientific, Hampton, New Hampshire, United States) using the Soxhlet apparatus for 16 hours. The solvent was evaporated under reduced pressure at 45°C using a rotary evaporator (Buchi R-210, Flawil, Switzerland) to give a yield of 8.4 % of dry extract. The dried *Zingiber officinale* rhizome ethanolic (ZORE) extract obtained was stored at -20 °C until further use. Then the extract was reconstituted with Dimethyl sulfoxide (DMSO) to produce the desired concentrations for further analysis.

### **2.3.Bacterial Strains**

The antibacterial activity was tested against *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis*, *Vibrio cholera*, *Vibrio parahaemolyticus*, *Pseudomonas aeruginosa* (ATCC 15442), *Enterotoxigenic Escherichia coli* (ETEC) (Resistant to Ampicillin, Chloramphenicol,

Gentamicin, Sulfamethoxazole, and Tetracycline) and *Salmonella typhi*. The above standard and clinically isolated strains were obtained from the Department of Biomedical Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. Cultures and morphological features of the strains were confirmed by biochemical characterization.

#### **2.4. Antibacterial activity**

All the chemicals used for media preparation and the antibiotics were purchased from Hi-Media Limited, Mumbai, India.

##### **2.4.1. Disc diffusion method**

The test organism were swabbed to the freshly prepared sterile Muller-Hinton agar plates. Sterile paper discs (6mm) were impregnated with various concentrations of the ZORE extracts (5-25 mg) suspended in DMSO and DMSO was used as negative control. The discs were dried to remove the DMSO solvent in a laminar air flow and were placed on the surface of the petri plates using sterile forceps. Plates were incubated at 37°C for 24 hours. Finally the zone of inhibition were noted and tabulated.

##### **2.4.2. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)**

Minimum inhibitory concentration (MIC) of ZORE extract was determined following Natta et al 2008. To perform MIC, the extracts were serially diluted so that the consecutive dilutions were half the concentration of the previous. Initial concentration of the ZORE extract was 20mg/ml which was diluted to obtain 10, 5, 2.5, 1.25 and 0.625mg/ml concentration of the extract. To each extract, 100µl of test organism were added, the tube without bacterial strain was kept as negative control. Tubes were incubated at 37°C for 24 hours, the MIC of ZORE extract

was determined by measuring the optical density in a spectrophotometer (620 nm), via comparing the sample readout with that of the non-inoculated nutrient broth. To determine the MBC, dilutions showing no visible growth in MIC was sub cultured in nutrient agar plate and incubates at 37°C for 24 hours. Plate showing no growth were noted for MBC.

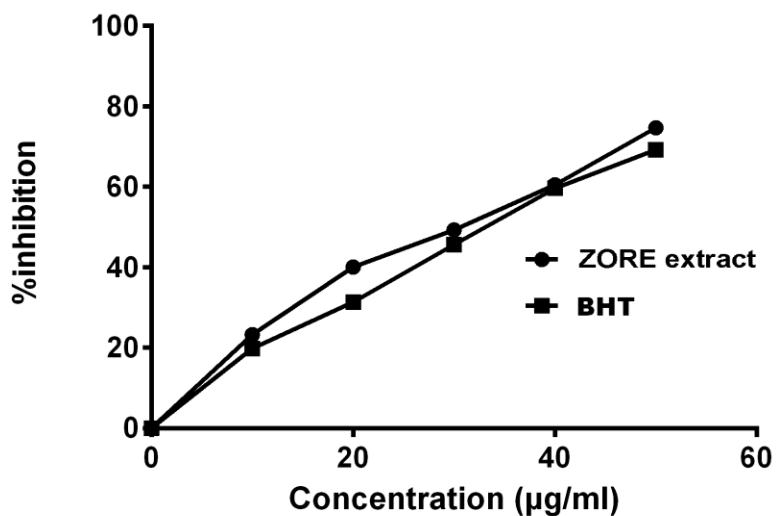
**Table1.** Zone of inhibition (mm) formed on various bacteria by different concentrations of ZORE extract in disc diffusion method

ZORE extract Concentration (µg/ml)	Zone of inhibition of various organisms (mm)						
	A	B	C	D	E	F	G
<b>50</b>	6	8	7	7	7	6	7
<b>100</b>	12	12	11	11	10	10	10
<b>150</b>	13	14	13	15	14	13	13
<b>200</b>	14	16	15	18	16	15	16
<b>250</b>	18	19	18	21	19	18	19

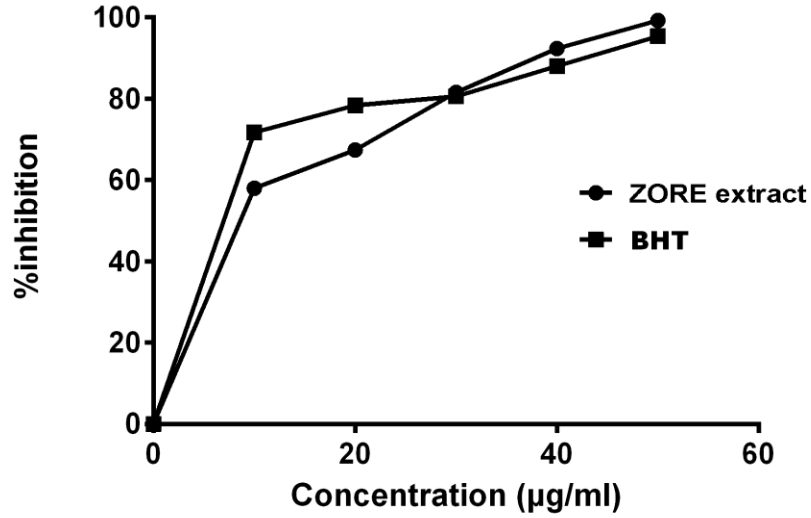
(A: *Staphylococcus aureus*, B: *Bacillus subtilis*, C: *Vibrio cholera*, D: *Vibrio parahaemolyticus*, E: *Pseudomonas aeruginosa*, F:ETEC and G: *Salmonella typhi*)

**Table 2.** MIC and MBC values of ZORE extract bacterial strains

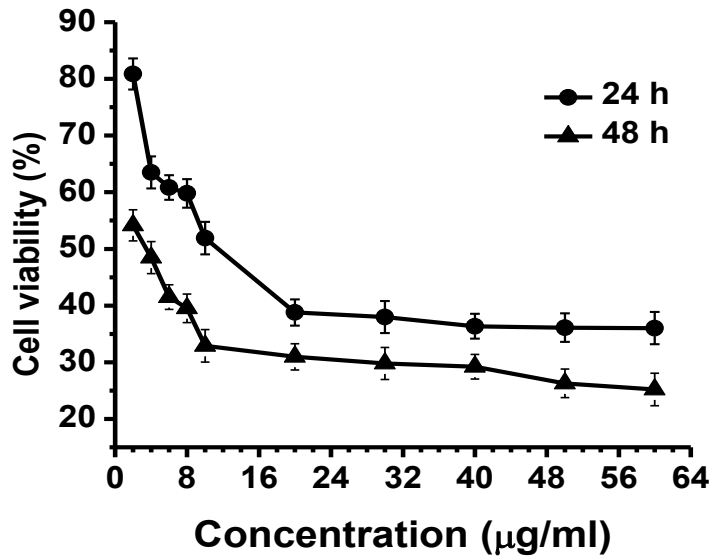
Microorganism	MIC (mg/ml)	MBC (mg/ml)
<i>Staphylococcus aureus</i>	0.625	1.25
<i>Bacillus subtilis</i>	0.625	1.25
<i>Vibrio cholera</i>	5	10
<i>Vibrio parahaemolyticus</i>	2.5	5
<i>Pseudomonas aeruginosa</i>	0.625	1.25
ETEC	1.25	2.5
<i>Salmonella typhi</i>	5	10



**Figure 1.** ABTS free radical scavenging assay of ZORExtract with butylated hydroxytoluene (BHT) control.



**Figure 2.** DPPH free radical scavenging assay of ZORE extract with butylated hydroxytoluene (BHT) control.



**Figure 3.** MTT assay of ZORE extract on MCF-7 cell line shown for 24 and 48 hours.

## 2.5. Freeradical scavenging activity

All the chemicals used for free radical scavenging activity were purchased from Hi-Media Limited, Mumbai, India.

### 2.5.1. 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS<sup>+</sup>) radical scavenging assay

ABTS<sup>+</sup> scavenging activity of ZORExtract was assayed following the method of Re et al., 1999. Seven mM ABTS<sup>+</sup> solution and 2.4 mM potassium persulfate solution was prepared as stock solution. The working solution was prepared by mixing equal quantities of each stock solution and allowing them to react in dark for 12 h at room temperature. The solution was then diluted by mixing 1 ml ABTS<sup>+</sup> solution with 60 ml methanol to obtain an absorbance of  $0.706 \pm 0.001$  units at 734 nm. Fresh ABTS<sup>+</sup> solution was prepared for each assay. Plant extract (1 ml) of various concentrations ranging from 20 to 100 mg were allowed to react with 1 ml of the ABTS<sup>+</sup> solution and the absorbance was taken at 734 nm after 7 min using UV Vis spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, Santa Clara, United States). The ABTS<sup>+</sup> scavenging capacity of the extract was compared with that of butylated hydroxytoluene (BHT) standard and percentage of inhibition was calculated using the below formula

$$ABTS^+ \text{ radical scavenging activity (\%)} = [(Abs \text{ control} - Abs \text{ sample}) / (Abs \text{ control})] \times 100$$

Where Abs control is the absorbance of ABTS radical + methanol; Abs sample is absorbance of ABTS radical + sample extract/standard. The sample concentration providing 50% inhibition (IC<sub>50</sub>) was calculated from the graph of inhibition percentage against sample concentration. All



determinations were carried out in triplicates. The ABTS radical-scavenging activity of BHT was assayed for comparison as positive control.

### **2.5.2. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay**

The effect of extract on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi, 2005. A solution of 0.135 mM DPPH in methanol was prepared and 1 ml of this solution was mixed with 1 ml of extract in ethanol containing 20 to 100 mg of the extract. The reaction mixture was vortexed thoroughly and left in dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm using a UV Vis spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, Santa Clara, United States). BHT was used as a reference standard. The ability to scavenge DPPH radical was calculated by the following formula.

$$DPPH \text{ radical scavenging activity (\%)} = [(Abs \text{ control} - Abs \text{ sample}) / (Abs \text{ control})] \times 100$$

Where Abs control is absorbance of DPPH radical + methanol; Abs sample is absorbance of DPPH radical + sample extract/standard. All determinations were carried out in triplicates.

### **2.6.MTT *in vitro* anticancer assay**

MCF-7 cell line in a monolayer containing approximately  $1 \times 10^4$  cells were added to each well of a 96 well plate containing Dulbecco's modified Eagle's medium and were incubated for 8h at 37°C in a CO<sub>2</sub> incubator (Galaxy® 170 S, Eppendorf, Hamburg, Germany) with a humidified atmosphere of 95% air and 5% CO<sub>2</sub>. After 8h incubation, cells were exposed to increasing concentrations of ZORE extract (2–50 µg/ml) and were incubated for 24 and 48 hours as above. After incubation 20 µl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to each well then the cultures were further incubated for 4 h, MTT was aspirated and

then 200 ml of DMSO was added to dissolve the formazan crystals. The absorbance was measured at 570 nm using microplate reader (Bio-rad, USA).

### 3. Results

#### 3.1. Antibacterial activity

The results of the antibacterial activity of ethanol extract on the various bacterial strains are as shown in table 1. The table indicated that the extracts showed slight variable degree of antibacterial activity on the different pathogens. The ZORE extract showed greatest inhibition on pathogen *Vibrio parahaemolyticus* with a zone of inhibition of 21 mm, in a concentration of 250 µg/ml. All the other organisms namely *Staphylococcus aureus*, *Bacillus subtilis*, *Vibrio cholera*, *Pseudomonas aeruginosa*, ETEC and *Salmonella typhi* exhibited a little less inhibition of 18 or 19 mm in the same concentration. The least inhibition was observed on *Staphylococcus aureus* and ETEC with a inhibition zone of 6 mm at a concentration of 50 µg/ml.

##### 3.1.1. MIC and MBCdetermination

The MIC and MBC values of all the pathogens for the ZORE extract is as tabulated in table 2. The least MIC value was observed for *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* which was 0.625mg/ml followed by ETEC with a MIC value of 1.25mg/ml. The highest MIC value for the ZORE extract was observed for *Vibrio cholera* and *Salmonella typhi* which was 5mg/ml, followed by *Vibrio parahaemolyticus* with a MIC value of 2.5 mg/ml.

The ZORE extract conferred good MBC even in a low concentration of 1.25 mg/ml for *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*, followed by ETEC with a MBC value of 2.5 mg/ml. *Vibrio cholera* and *Salmonella typhi* expressed the highest MBC

value of 10mg/ml for the ZORE extract. None of the studied bacteria namely *Staphylococcus aureus*, *Bacillus subtilis*, *Vibrio cholera*, *Vibrio parahaemolyticus*, *Pseudomonas aeruginosa*, ETEC and *Salmonella typhi* were able to withstand high MBC 10 mg/ml of the ZORE extract.

### **3.2.Freeradical scavenging activity**

#### **3.2.1. ABTS radical scavenging activity**

The principle behind the technique involves the reaction between ABTS and potassium persulphate to produce the ABTS radical cation, a blue green chromogen. The presence of antioxidant-reductant, the colored radical is converted back to colorless ABTS, the absorbance of which is measured at 734 nm. The IC<sub>50</sub> value of the ZORE extract and BHT to scavenge the ABTS radical is approximately 30 µg/ml and 35µg/ml respectively, where BHT is the reference standard (Figure 1).

#### **3.2.2. DPPH radical scavenging activity**

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The degree of discoloration indicated the scavenging potential of the antioxidants in the extract. IC<sub>50</sub> value of the ZORE extract and BHT to scavenge the DPPH radical is less than 10 µg/ml and 10 µg/ml respectively, where BHT is the reference standard (Figure 2).

### **3.3. MTT *in vitro* anticancer assay**

MTT assay was performed to determine the cytotoxic effect of ZORE extract on MCF-7 cells. The ZORE extract effectively inhibited proliferation of MCF-7 cells in a dose-dependent manner over 24 h (Figure3). The IC<sub>50</sub>value of the ZORE extract was 10 µg/ml. DMSO was used as control and did not have any anti-proliferation effecton the MCF-7 cell line.

#### 4. Discussion

In today's scenario most of the pathogens have developed resistant to the main stream antibiotics, therefore there arises a need for new antibiotics. Plants have been the most valuable source for natural product to maintain human health and their constituents confer good antimicrobial activity (Sinha *et al.*, 2013). According to world health organization medicinal plants have been the cradle for good antimicrobial and antiseptic compounds. In most developing countries 60 to 90% of the people used medicinal plants in their daily dietary (WHO 2002). Presence of phytochemical components such as flavonoids, phenolic components and tannins plays a vital role in the antibacterial activity of plant extracts (Barnabas and Nagarajan 1988 and Burapedjo and Bunchoo 1995). In this study, we used ethanol as solvent for extraction of compounds from ginger rhizome and compare its activity against several human bacterial pathogens. There are many investigators in the past who suggested that plant extracts of different solvents to have great potential of antibacterial activity in comparison to aqueous extract (Leelarasamee *et al.*, 1990 and Voravuthikunchai and Limsuwan, 2006). In our study, the ethanolic extract of *Zingiber officinale* rhizomes showed great anti-bacterial activity against human bacterial pathogens such as *Staphylococcus aureus*, *Bacillus subtilis*, *Vibrio cholera*, *Vibrio parahaemolyticus*, *Pseudomonas aeruginosa*, *ETEC* and *Salmonella typhi*.

The *Z. officinale* rhizome ethanolic (ZORE) extract demonstrated adequate to strong antibacterial activity, and showed stronger inhibitory effects against all studied human pathogens ( $p < 0.05$ ). The results inferred that the most sensitive bacteria were the standard strains namely of *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*.

The effect of ethanolic extracts of ginger were earlier studied by Bhargava et al. (2012), and have demonstrated similar MIC values for the ethanolic extract against the standard test bacteria (*Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*). Taura et al. (2014) have showed that the ethanolic extract of ginger was more effective on *S. aureus* (MIC at 100 µg/ml), but it did not act against *E. coli* and *Pseudomonas aeruginosa* but in the current study we have found results in contradiction to them but conforming to another study done by Naji and Jassemi (2010) on the ethanolic extract of ginger showed the best effect against *Pseudomonas aeruginosa* and *E. coli*. In our study, ethanolic extract showed the strongest effect on *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. Apart from this in the current study the ethanolic extract of ginger has shown acceptable activity against common human pathogens such as *Vibrio cholera*, *Vibrio parahaemolyticus*, ETEC and *Salmonella typhi* providing a new lead.

Natural antioxidants have inordinate impact on the safety of the food system and will continue to do so. Not only do they keep the food stable against oxidation but can also be effective in controlling microbial growth (Stoilova et al., 2007). Ginger is an indispensable component of many food additives. The current study showed that the ginger rhizome ethanolic extract can control the quantity of free radicals very effectively. Thus, it could prevent or decrease the damage in a human body caused by free radicals, which, according to Aruoma et al., 1997 and Valko et al., 2004, attack biological macromolecules such as lipids, proteins and DNA. By averting the oxidation of the phospholipids of cell membranes, the specific cell permeability is preserved and the cell metabolism is not disturbed. Though all organisms retain antioxidant mechanisms for protection from oxidative damage, it is insufficient to prevent all possible

damage. This is the reason for the interest towards the inclusion of nontoxic antioxidant flavanoids and polyphenols in the human diet which are abundant in ginger. Apart from the studied antiradical activity, ginger rhizome ethanolic extract was also subjected to *in vitro* toxicology to ascertain its effectiveness against breast cancer cell line MCF-7.

*Zingiber officinale* is seen as an important medicinal plant used in traditional medicine to cure various diseases in South Asia (Shukla and Singh, 2007). The results of the present study indicated that the ethanol extract of ginger rhizome is potent in inhibiting the proliferation of ER-positive breast cancer cells (MCF-7). The results of the current study demonstrate that ginger not only has a powerful inhibitory effect on the proliferation of ER-positive (MCF-7) breast cancer cells but also contains significant antibacterial and antioxidant activity. The findings hold promise for further *in vitro* and *in vivo* molecular target-oriented studies to examine the chemoprotective efficacy of ethanol extract of ginger rhizome, particularly for ER-negative breast cancers, which have a poorer prognosis and shorter survival (Skildum et al, 2002).

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