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Evaluation of Various Crude Extracts of *Zingiber officinale* Rhizome for Potential Antibacterial Activity: A Study *in Vitro*

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Abstract

In vitro antibacterial activity of crude aqueous and organic extracts of rhizome of *Zingiber officinale* Roscoe (ginger) was studied against both Gram-negative (*Escherichia coli* and *Salmonella typhi*) and Gram-positive (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus pyogenes*) bacterial strains. The present study reveals that the pattern of inhibition varied with the solvent used for extraction and the organism tested. Plant extracts prepared in organic solvents provided more consistent antibacterial activity as compared to aqueous extracts. Methanol extract was the most active against maximum number of bacterial species tested. Gram-positive bacteria were found the most sensitive as compared to Gram-negative bacteria. *Staphylococcus aureus* was significantly inhibited by almost all the extracts even in very low MIC followed by other Gram-positives. *Escherichia coli* (a Gram-negative bacterium) was showing the least inhibition with highest MIC values, while *Salmonella typhi* was found completely resistant. Methanol extract yielded the presence of terpenoids, flavonoids, alkaloids and tannins in phytochemical screening. Results of the present study sign the interesting assurance of designing a potentially active antibacterial agent from *Zingiber officinale*.

Keywords: *Zingiber officinale*, Antibacterial Activity, Agar-Well Diffusion Assay, Minimum Inhibitory Concentration, Microbroth Dilution, Phytochemistry

1. Introduction

Since the introduction of antibiotics, there has been tremendous increase in the resistance of diverse bacterial pathogens [1,2]. Several species of plants have been used for centuries as remedies for human diseases because these contain components of therapeutic values [3,4]. Recently, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led researchers to investigate antimicrobial activity of medicinal plants [5-12].

Zingiber officinale Roscoe is a common household spice originated from Southeast Asia; a city with its Sanskrit name *Shunti* was already in existence in 200 B. C. Ginger is also called as "*The Great Medicament*" in Ayurvedic medicines [13]. It belongs to family Zingiberaceae and is a perennial plant with thick tuberous rhizomes (**Figure 1(a)**), which are the medicinally useful

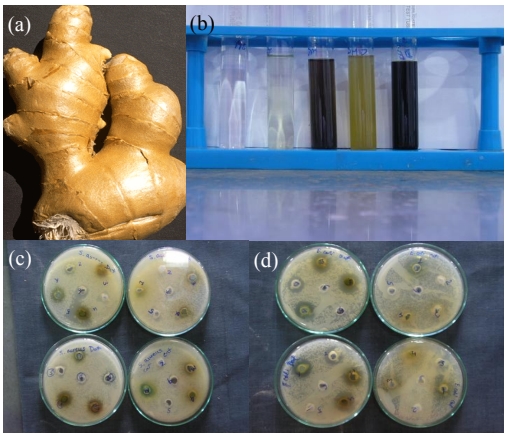

Figure 1. Antibacterial potential of crude extracts of rhizome of *Zingiber officinale* (a) Rhizome of Ginger; (b) Dry powder extracts prepared in different solvents; (c) Zone of inhibition of different extracts against Gram-positive *Staphylococcus aureus*; (d) Zone of inhibition of different extracts against Gram-negative *Escherichia coli*.

part of this plant. The medicinal history of ginger has been extensively searched throughout the world and found to possess anti-inflammatory, cholesterol-lowering, and antithrombotic properties [13,14]. Important secondary metabolites present in the rhizome are curcumene, non-volatile hydroxyaryl compounds e.g. zingerone, gingeroles and shogaoles (phenylalkanones), volatile sesquiterpenes (e.g. zingiberene and bisabolene) and monoterpenoids (e.g. citral) [15]. Although, the antimicrobial activity and chemical analysis of essential oil and olioresins of this plant has been investigated [16], the present study was focussed to investigate the antibacterial potential of crude extracts of rhizome of *Zingiber officinale*. Furthermore, active extracts were evaluated for their minimum inhibitory concentrations (MICs) and phytochemical screening.

2. Materials and Methods

2.1. Collection of Plant Part

Plant part (rhizome) of *Zingiber officinale* was collected during winter between September 2006 and January 2007. Rhizome was first washed under running tap water followed by sterilized distilled water, air-dried and then powdered with the help of sterilized pestle and mortar. This powder was stored in airtight bottles and subjected to various extraction procedures.

2.2. Preparation of Crude Extracts of Ginger Rhizome

Following methods were applied in preparation of crude extracts of rhizome of *Zingiber officinale* (**Figure 1(b)**).

2.2.1. Aqueous Extraction

To make aqueous decoction, air-dried powder of plant part (10 g) was boiled in 400 ml distilled water till one fourth of the extract initially taken was left behind after evaporation. The solution was then filtered using muslin cloth. Filtrate was centrifuged at 5000 rpm for 15 min. The supernatant was again filtered using Whatman Filter No. 1 under strict aseptic conditions and the filtrate was collected in fresh sterilized bottles and stored at 4˚C until further use.

2.2.2. Organic Solvent Extraction

Air-dried powder (10 g) was thoroughly mixed with 100 ml organic solvent (viz. ethanol, methanol, hexane and ethyl acetate). The mixture was placed at room temperature for 24 h on shaker with 150 rpm. Solution was filtered through muslin cloth and then re-filtered by passing through Whatman Filter No. 1. The filtrate thus obtained was concentrated by complete evaporation of solvent at room temperature to yield the pure extract. Stock solutions of crude extracts for each type of organic solvent were prepared by mixing well the appropriate amount of dried extracts with respective solvent to obtain a final concentration of 100 mg/ml. Each solution was stored at 4˚C after collecting in sterilized bottles until further use.

2.3. Bacterial Strains Selected for Susceptibility Assay

A total of six bacteria namely *Escherichia coli* MTCC-739, *Salmonella typhi* MTCC-531 (all Gram-negative bacteria) and *Bacillus cereus* MTCC-430, *Bacillus subtilis* MTCC-736, *Staphylococcus aureus* MTCC-740 and *Streptococcus pyogenes* MTCC-442 (all Gram-positive bacteria) were screened for present investigation. All the above mentioned bacterial strains were collected from Microbial Type Culture Collection (MTCC), India. These bacterial cultures were maintained in nutrient agar slants at 37˚C. Each of the microorganisms was reactivated prior to susceptibility testing by transferring them into a separate test tube containing nutrient broth and incubated overnight at 37˚C.

2.4. Antibacterial assay

Antibacterial activities of all aqueous and organic extracts of rhizome of *Zingiber officinale* were determined by standard agar well diffusion assay [17]. Petri dishes (100 mm) containing 18 ml of Mueller Hinton Agar (MHA) seeded with 100 µl inoculum of bacterial strain (inoculum size was adjusted so as to deliver a final inoculum of approximately 10^8 CFU/ml). Media was allowed to solidify and then individual Petri dishes were marked for the bacteria inoculated. Wells of 6 mm diameter were cut into solidified agar media with the help of sterilized cup-borer. 50 µl of each extract was poured in the respective well and the plates were incubated at 37˚C for overnight. Organic solvents were used as negative control while tetracycline antibiotic (5 $\mu g \cdot ml^{-1}$) was used as positive control. The experiment was performed in triplicate under strict aseptic conditions and the antibacterial activity of each extract was expressed in terms of the mean of diameter of zone of inhibition (in mm) produced by the respective extract at the end of incubation period.

2.5. Determination of Minimum Inhibitory Concentration

Extracts producing an inhibition zone ≥12 mm in diameter were screened to determine minimum inhibitory con-

centrations (MICs) by standard two-fold microbroth dilution methodology given by NCCLS [18]. A stock solution of each extract (viz. ethanol, methanol, ethyl acetate and aqueous) was serially diluted in 96-wells microtiter plate with Mueller Hinton broth to obtain a concentration ranging from 8 µg/ml to 4096 µg/ml. A standardized inoculum for each bacterial strain was prepared so as to give inoculum size of approximately 5×10^5 CFU/ml in each well. Microtiter plates were then kept at 37˚C for an overnight incubation. Following incubation, the MIC was calculated as the lowest concentration of the extract inhibiting the visible growth of bacterial strain.

All the chemical ingredients used in present study were of analytical grade, and were purchased from Hi Media, India.

2.6. Phytochemical Analysis of Active Crude Extract

Methanol extract of ginger rhizome was evaluated for its phytochemistry by standard methodology as given by Harborne [19].

3. Results and Discussion

Table 1 represents the antibacterial activity of various crude extracts prepared from the rhizome of ginger. Data indicated that extracts prepared in organic solvents consistently displayed better antibacterial activity than that of aqueous extracts. Extracts prepared in methanol was observed most inhibitory (diameter of zone of inhibition ranging from 12.83 to 18.67 mm) followed by those prepared in ethyl acetate (zone of inhibition 10.33 to 14.00 mm). Ethanol and hexane extract was found mild inhibitory only against *Staphylococcus aureus* with only 13.66 and 10.33 mm diameter of zone of inhibition, respectively. The zone of inhibition observed with aqueous extract was 15.67 mm against Gram-positive staphylococci.

All antimicrobial activities observed varied with the type of test organism. Primary screening indicated that extracts were more effective in inhibiting Gram-positive bacteria when compared to Gram-negative bacteria. *Staphylococcus aureus* (a Gram-positive bacterium) was significantly inhibited by almost all the extracts and found most susceptible among all the bacterial species examined in this study (**Figure 1(c)**). *Bacillus cereus* and *Bacillus subtilis* were also inhibited but comparatively smaller zone of inhibitions were obtained.

Ginger extracts had a very little effect on Gram-negative bacteria specifically against *Escherichia coli* (**Figure 1(d)**); however, the Gram-negative bacterium; *Salmonella typhi* and Gram-positive *Streptococcus pyogenes* demonstrated the complete resistance against all the extracts as no zone of inhibition was observed. Our results were found in agreement with some earlier studies which showed the moderate antibacterial properties of ginger extract against *Escherichia coli* but no activity was

Table 1. *In vitro* antibacterial activity of aqueous and organic extracts of *Zingiber officinale* rhizome.

Type of Extract			Zone of Inhibition* (in mm diameter)					
			Gram-negative Bacteria		Gram-positive Bacteria			
			Escherichia coli	*Salmonella typhi*	*Streptococcus pyogenes*	*Staphylococcus aureus*	*Bacillus subtilis*	*Bacillus cereus*
Organic Extract		Ethanol	NI	NI	NI	13.66 ± 0.29	NI	NI
		Methanol	12.83 ± 0.76	NI	NI	18.67 ± 1.52	14.00 ± 1.00	13.00 ± 1.32
		Ethyl Acetate	10.33 ± 0.58	NI	NI	14.00 ± 2.00	11.34 ± 0.58	11.16 ± 0.77
		Hexane	NI	NI	NI	10.33 ± 0.58	NI	NI
Aqueous Extract			NI	NI	NI	15.67 ± 2.08	NI	NI
Control	Positive	Tetracycline+	29.50 ± 0.50	25.83 ± 1.61	29.83 ± 1.89	32.50 ± 1.50	34.17 ± 1.76	32.16 ± 1.04
	Negative	Ethanol	NI	NI	NI	NI	NI	NI
		Methanol	NI	NI	NI	NI	NI	NI
		Ethyl Acetate	NI	NI	NI	NI	NI	NI
		Hexane	NI	NI	NI	NI	NI	NI

*Values of the observed zone of inhibition (in mm diameter) including the diameter of well (6 mm) after 24 hours incubation against different bacterial species when subjected to different extracts in agar well diffusion assay. Assay was performed in triplicate and results are the mean of three values ± Standard Deviation. In each well, the sample size was 100 µl. Inhibition observed in extracts due to solvent were assessed through negative controls. "NI"—No Inhibition Zone was observed. +Tetracycline (5 $\mu g \cdot ml^{-1}$) was used as standard antibiotic.

observed against *Salmonella* and some other bacterial species [20,21]. Although, some studies were carried out to evaluate the antimicrobial activity and chemical analysis of essential oil and olioresins of this plant against various oral and food-borne bacterial and fungal pathogens [16,22]; however, the present study was focussed mainly to investigate the antibacterial potential of crude extracts of rhizome of *Zingiber officinale* in terms of MIC against pathogenic bacteria.

Control experiments using standard solvents used for extract preparation (*i.e.* negative control) showed no inhibition of any bacteria, indicating that raw ginger itself and not solvent inhibited the growth of the Gram-positives and Gram-negatives. Tetracycline (a positive control) showed variable inhibition diameters ranging from 25.83 to 34.17 mm against Gram-positive and Gram-negative bacteria.

Active extracts thus obtained (methanol, ethyl acetate, ethanol and aqueous extracts) were subjected to determine minimum inhibitory concentration (MIC) by two-fold microbroth dilution method against respective susceptible bacterial species (**Table 2**).

The results indicated that methanol extract was found most significant inhibitor than other extracts and this extract inhibited the *Staphylococcus aureus* comparatively at very lower concentration of 512 µg/ml. *Bacillus subtilis* was inhibited by this extract at 4096 µg/ml; however, comparatively higher inhibitory concentrations were required for inhibition of both *Bacillus cereus* and *Escherichia coli*. The next potent inhibitor was aqueous extract with MIC 2048 µg/ml for Gram-positive *Staphylococci*. MICs for ethanol and ethyl acetate extracts were seen comparatively higher. Furthermore, Gram-positive bacterial species were found most sensitive as compared to Gram-negatives. Methanol extract was interpreted as most significant inhibitory against bacterial species evaluated, thus screened for phytochemical analysis by the standard methodologies given by Harborne. Phytochemical analysis revealed the presence of terpenoids, flavonoids, alkaloids and tannins in this extract (**Table 3**).

Predictions of antimicrobial activity in herbal compounds extracted from plant parts depend largely upon the type of solvent used for extraction. Traditional practitioners have used water as the primary solvent in extraction of herbal compounds since Vedic times (earlier than 6000 BCE); however, the present study reveals that the use of organic solvents in the preparation of plant extracts provides more consistent antibacterial ac- tivity as compared to aqueous extracts. The reason be- hind this can be given in terms of higher solubility of bacterial cell wall peptidoglycan and lipopolysaccharide layers in organic solvents. This finding is in agreement with the earlier study done by Tan and Vanitha [13]. Furthermore, methanol extract was found to be better inhibitory than that of ethanol extract. The study was supported by the findings of Nanasombat and Lohasuthawee [23]; as they also found the relatively lesser activity of ethanol extract.

This observation clearly indicates that the polarity of antibacterial compounds make them more readily extracted by organic solvents, and using organic solvents does not negatively affect their bioactivity against bacterial species. The data also showed that some antimicrobial substances could only be extracted by organic solvents, suggesting that organic solvents are clearly better solvents of antimicrobial agents [24].

Furthermore, Gram-positive bacteria were found more susceptible than Gram-negative bacteria. *Staphylococcus aureus* (a Gram-positive bacterium) was observed as most susceptible bacterium in present study which is in agreement with the study of Chen *et al.* [25]. This is probably due to the differences in chemical composition and structure of cell wall of both types of microorganisms.

Table 2. Minimum inhibitory concentration of active crude extracts of *Zingiber officinale* rhizome.

Type of Active Crude Extract	Test Microorganism	Concentration of Extracts* (in µg·ml^{-1})										MIC (in µg·ml^{-1})
		4096	2048	1024	512	256	128	64	32	16	8	
Ethanol	*Staphylococcus aureus*	–	+	+	+	+	+	+	+	+	+	4096
Methanol	*Escherichia coli*	+	+	+	+	+	+	+	+	+	+	>4096
Methanol	*Staphylococcus aureus*	–	–	–	–	+	+	+	+	+	+	512
Methanol	*Bacillus subtilis*	–	+	+	+	+	+	+	+	+	+	4096
Methanol	*Bacillus cereus*	+	+	+	+	+	+	+	+	+	+	>4096
Ethyl Acetate	*Staphylococcus aureus*	–	+	+	+	+	+	+	+	+	+	4096
Aqueous	*Staphylococcus aureus*	–	–	+	+	+	+	+	+	+	+	2048
Tetracycline	*Staphylococcus aureus*	–	–	–	–	–	–	–	–	–	–	<8

*Different concentrations of active crude extracts evaluated in 96-well microtiter plate using Microbroth Dilution Assay as recommended by NCCLS. All values are expressed in µg·ml^{-1}; (–) represents "No Growth Observed"; (+) represents "Growth Observed".

Table 3. Phytochemical analysis of methanol extract of *Zingiber officinale* rhizome.

Test	*Zingiber officinale* Methanol Extract
Terpenoids	+
Steroids	–
Flavonoids	+
Alkaloids	+
Tannins	+
Saponin	–

All antimicrobial activities occurred in a concen- tration-dependent manner, however, the efficacy of extracts are lesser than to that of standard antibiotic, tetracycline.

The results of the present study clearly indicate the antibacterial potential in the rhizome of *Zingiber officinale* (ginger). Furthermore, active plant extracts can be subjected to various pharmacological evaluations by several methods such as NMR, GC-MS etc. for the isolation of the therapeutic antimicrobials.

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