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IN VITRO ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *ZINGIBER OFFICINALE*

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ABSTRACT

Zingiber officinale commonly called Ginger, Agat, Gengibre, or Luya, family-Zingiberaceae is a world-wide species. Commonly the rhizome of this plant use as the source of spices,ginger tea preparation, ginger lozenges and flovoring of confectionaries,ginger ale, ginger beer, ginger champagnes, and other beverages.The research work was designed to investigate the antimicrobial activity and anti-oxidant activity of *Zingiber officinale.* Anti-microbial activity of methanol extract of *Zingiber officinale* was tested by disc diffusion method.Standard antibiotic disc of kanamycin(30µg/disc) for bacterial species were used as standard and crude extracts were used at a concentration of 500µg/disc.The extract of rhizome posses significant anti-bacterial activity with average zone of inhibition11-19mm. Maximum antibacterial property shown against *Salmonela paratyphii* having the zone of inhibition of 19mm.The test signifies that the MIC of the methanol extract of the rhizome was 128µg/ml against *salmonella paratyphii*. The free radical scavenging activity of methanol extract of rhizome of *Zingiber officinale* was evaluated in vitro with the spectrophotometric method based on the redction of the stable DPPH freee radical.Ascorbic acid was used as a reference standard.The IC50 value of methanol extract *Zingiber officinale* was found 239.16 µg/ml.

KEYWORDS: Aantimicrobial, Antioxidant, DPPH, Disc diffusion method.

INTRODUCTION

The medicinal plants are not only use in medicine but also in cosmetics, detergents, dyes, chemical, insecticides, foods and paints etc. According to world health organization (WHO), medicinal plants are an accessible, affordable and culturally appropriate source of primary health care for more than 80% of Asia's population.

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesis hundreds of chemical compounds for functions including defence against insects, fungi, diseases, and herbivorous mammals. Numerous phytochemicals with potential or established biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain. Further, the phytochemical content and pharmacological actions, if any, of many plants having medicinal potential remain unassessed by rigorous scientific research to define efficacy and safety. In the United States over the period 1999 to 2012, despite several hundred applications for new drug status, only two botanical drug candidates had sufficient evidence of medicinal value to be approved by the Food and Drug Administration.

It has been known from ancient times that essential oils from aromatic and medicinal plants possess biological activity, antibacterial, antifungal and antioxidant properties. Due to the growing interest in the use of essential oils in both the food and the pharmaceutical industries, a systematic study on these plant extracts have become very important. Spices were used from ancient times for different purposes flavoring, keeping away the pests, and inperfumery. Ginger is a rhizomatous plant grown throughout South-eastern Asia, China and in parts of Japan, Austria, Latin America, Jamaica and Africa. Ginger has been used as a spice and medicine in India and China since ancient times. Ginger plants were grown in pots and carried to abroad on sea long voyages to prevent scurvy. The spice was known in Germany and France in the ninth century and in England in 10^{th} century for its medicinal properties. Many oils exhibit antimicrobial properties due to the presence of components such as thymol, eugenol, 1,8-cineole, α- and β- pinenes, linalool, α-terpineol etc1,2. Since these compounds and their relative concentration vary from oil to oil and from different oils which accounts for a varied antimicrobial activity 3. Over three quarters of the world population still rely on plants and plant extracts for health care. Ginger is widely used in ayurvedic medicines and in

folklore medicines1. About 8000 herbal remedies have been codified in ayurveda and are still used throughout India. Most of the ayurvedic preparations contain dry pepper and ginger.

Ginger contains 1-2 % oil, which imparts the unique flavour to the spice and it has been studied by many workers 4-9. Many reports are available on the chemical composition of fresh ginger oil and the naturally occurring flavouring compounds 10-12.Some reports are available on the antimicrobial property of the volatile oil from the rhizomes of ginger 13-20. The essential oil from ginger, was studied for antimicrobial activity against Aspergillusniger, Saccharomyces cerevisiae, Mycoderma sp., Lactobacillus acidophilus and Bacillus cereus, as determined by paper agar diffusion method 20.Another study 21reports on the bioassay-guided isolation of antifungal compounds from an African land race of ginger, *Zingiber officinale*

Roscoe, and the identification of 6, 8 and 10-gingerols and 6-gingerdiol as the main antifungal principles. The compounds were active against 13 human pathogens at concentrations of <1 mg/mL. The gingerol content of the African land race was at least 3 times higher than that of typical commercial cultivars of ginger 21. A survey of the literature reveals that there are no reports on the antimicrobial properties of the fresh ginger oil on the selected microorganisms. So this study was carried out.

METHOD AND MATERIALS

Collection of the plant parts

The fresh rhizome of *Zingiber officinale* were selected for the study. The rhizome of these plants were collected during month of february, 2017 from the area of Raipura, Narsingdi, Bangladesh and were identified by the experts of Bangladesh National Herbarium, Dhaka, where voucher specimen were retained.

Drying, Pulverization and Preservation of plant parts

The rhizome were first washed with water to remove the adhering dirt and then cut into small pieces, sun dried for 12-15days. After complete drying, the entire portions were pulverized into coarse powder with the help of grinding machine and were stored in an air tight container for use.

Extraction of Plant Material

The each rhizome 100gm were extracted with 3 times methanol of their weight in a round bottom flask container with 1:2 sample and solvent ratio at room temperature through occasional shaking and stirring for 7 days. After 7 days, the extracts were filtered through filter paper. The filtrates were concentrated at 50ºC under reduce pressure in a rotary evaporator to afford a greenish mass of biological investigation. Then the crude extract ready for assaying of antimicrobial, antioxidant, total phenolic content.

Extraction procedure

In our current study we used cold extraction method.

Cold Extraction for the four plant parts Preparation of methanolic extracts For each plant the dried and powdered materials (500 g for) were soaked in 2500 ml of 90% ethanol for about 15 days at room temperature with occasional stirring. After 15 days the solution was filtered using filter cloth and Whatman's filter paper. The filtrates (Ethanolic extract) obtained were evaporated under rotary evaporator and in a water- bath until dried. It rendered a gummy concentrates and were designated as crude extracts of Ethanol.

Antimicrobial testing methods

The following three methods have been shown to consistently provide reproducible and repeatable results when followed correctly. They are- Disk diffusion, Broth dilution & Agar dilution.

Disc diffusion method Principle

In this method, measured amount of the test samples are dissolved in definite volumes of solvent to give solutions of known concentrations (µg/ml). Then sterile filter paper discs (5mm in diameters) are transfered in the petridish andapplied test sample in the disc by micropipette with known amounts of the test substances and dried. These plates are kept at low(4c) temperature in the refrigerator for 24 hours to allow maximum diffusion. The plates are then kept in an incubator (37ºc) for 12-18 hours to allow the growth of the organisms. If the test material has antimicrobial activity, it will inhibit the growth of microorganisms, giving a clear, distinct zone called "zone of inhibition".

The principal factors which determine the size of the zone of inhibition are.

Intrinsic antimicrobial susceptibility of the test sample. Growth rate of the test organisms. Diffusion rate of the test sample which is related to its water solubility. Concentration of the test organisms inoculated in the medium. Concentration of the test sample per disc.Thickness of the test medium in the petridishes.

MATERIALS

In our present study, the antimicrobial activity of distil water extract of *Zingiber officinale* rhizomes was investigated in comparison with standard Kanamycin (30µg/disc) antibiotic against a number of pathogenic gram-positive and gram-negative bacteria.

Table 1: Gram positive and gram-negative bacteria.

Gram-positive	Gram-negative
Staphylococcus aureus	*Escherichia coli*
Bacillus megaterium	*Shigella dysenteriae*
Bacillus subtilis	*Salmonella typhi*
Sarcina lutea	*Salmonella paratyphi*
	Vibrio cholerae
	Proteus vulgaris
	Klebsiella pneumoniae

Apparatus and Reagents

Filter paper discs (5mm in diameter), Petri dishes, Refrigerator, Test tubes, Sterile forceps, Sterile cotton, Incubating loop, Bunsen burner, Micropipette(10-100µl), Laminar air flow unit (Biocraft's Scientific Industries, India), Autoclave, Incubator(OSK-9636, Japan), Nutrient agar media(DIFCD), Methanol, Standard disc(Kanamycin 30 µg/disc).

Agar media

Agar is a gelatinous substance, obtained from algae. Agar is derived from the polysaccharide agarose, which forms the supporting structure in the cell walls of certain species of algae, and which is released on boiling. Throughout history into modern times, agar has been chiefly used as an ingredient in desserts throughout Asia and also as a solid substrate to contain culture media for microbiological work.

Composition of Agar media

5.0 (g/l), Peptone, 5.0 (g/l), beef extract/yeast extract, 15(g/l), agar, 5.0 (g/l), NaCl, distilled water, pH adjusted to neutral (68 g/l) at 25°C.

Procedure Preparation of Medium

To prepare agar media solution 28grams of agar has to be mixed with 1L of water. In case of preparation of 250ml agar media solution 7grams of agar has to be mixed with 250ml distil water. Then it is transfer to autoclave for sterilization for 20 minutes at 121ºC.

Preparation of subculture

With the help of an inoculating loop, the test organisms were transferred from the pure culture to the agar slants under a laminar air flow unit. The inoculated slants were then incubated at 37ºc for 18-24 hours to ensure the growth of the test organisms.

Preparation of test plates

The agar media is taken in test tube after autoclaving and the organism was transferred from the subculture to the test tube containing autoclaved medium with the help of an inoculating loop in an aseptic area. The test tube was shaken by rotation to get a uniform suspension of the organism.

Preparation of Discs

There are three types of disc available. They are Sample discs, Standard discs & Blank/control discs.

Sample discs

Sterilized filters paper discs (5 mm in diameter) were taken in a blank petridish. Sample solution of the desired concentration was applied on the discs with the help of a micropipette in an aseptic condition.

Standard discs

These were used to compare the antibacterial activity of test material. In our investigation,

Kanamycin (30µg/disc) standard disc was used as a reference.

Blank/control discs

Only solvent was applied to the discs to determine the antibacterial effects of the solvent used. But in these study we use water as a solvent so that blank or control disc is not required.

Preparation of Discs

Containing Sample Prepare the disc and then it transferred to the petridish by the help of a forcep. Then drug or sample is transferred in the disc by micropipette.

Measurement of Zone of Inhibition

After 18 hours of incubation, the antibacterial activity of the test samples was determined by measuring the diameter of inhibitory zones in term of millimeter.

Determination of antimicrobial activity by measuring zone of inhibition

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition.

METHOD OF ANTIOXIDANT

Principle

The free radical scavenging activities (antioxidant capacity) of the plant extract on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of brand – Williams et al., 1995. 2.0 ml of a methanol solution of the extract at different concentration were mixed with 3.0 ml of a DPPH methanol solution (20µg/ml). the antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that of tert-butyl-1-hydroxytoluene (BHT) by UV spectrophotometer.

DPPH was used to evaluated the free radical scavenging activity (antioxidant potential) of various compounds and medicinal plants (Choi et al., 2000; Desmarchelier e al, 2005). So, DPPH is used as reagents. DPPH is a stable free radiacls potentially reactive activity of specific compounds of extracts (Dinis, Madcria, Almeida, 1994). Because of its odd electron. DPPH has a strong absorption band at 517 nm. Since this electron becomes paired in the presence of a free radical scavenger, the absorption decrease stoichiometrically with respect to the number of electrons taken up. This change in absorbance produced by this reaction has been widely used to test the ability of several molecule to act as free radical scavengers (Dinis, Maderia, Almeida, 1994).

The absorbance was taken by UV-spectrophotometer and methanol was taken for extraction ans as solvent.Ascorbic acid was used as a standard.

Materials

1,1-diphenyl-2-picrylhydrazyl,Distilled water, Methanol., UV-spectrophotometer, Beaker, Teste tube, Light-proof box., Pipette(5ml), Micropipette(10-100 µl), Amber reagent bottle.

Method

2.0 ml of a methanol solution of the extract at different concentration (500 to 0.977 ug/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml).After 30 min reaction period at room temperature in dark place the absorbance was measured against at 517 nm against methanol as blank by UV spectrophotometer.

Inhibition free radical DPPH in present (1%) was calculated as follows:

$$. (1\%) = (1-A_{sample}/A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test material).

Extract concentration providing 50% inhibition (LC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration. Ascorbic Acid was used as positive control.

Tests carried out in triplicate and average value was taken.

The experiments were performed trice and results were expressed as mean ± SD in every case.

RESULT AND DISCUSSION

The results of antimicrobial activity of crude extracts against a number of gram positive and gram negative bacteria are given as below in the table.

Table 2: Diameter of the zone of inhibition in (mm) of *Zingiber officinale* extract.

Name of the organism	Type of the Extract	
	Methanol (500µg/disc)	Kanamycin Disc(30µg/disc)
Gram positive	Zone of Inhibition (mm)	
Bacillus subtilis	11	29
Bacillus megaterium	11	30
Bacillus cereus	15	32
Staphylococcus aureus	17	36
Sarcina luteae	15	34
Gram negative		
Salmonela paratyphi	19	35
Vibrio parahaemolyticus	15	32
Vibrio mimicus	14	30
Shigella boydii	12	28
Shigella dysenteriae	12	31
Escharichia coli	11	32
Pseudomonas aeruginosa	12	34
Fungus		
Candida albicans	11	28
Asperagillus niger	13	29
Sacharomyeces cereveceae	15	31

The methanol extraxts (500µg/disc) of the rhizome of Zingiber officinale was screened against 15 human pathogenic bacteria to check antibacterial activities by disc diffusion method. Here Kanamycin disc (30 µg/disc) used as reference standard.

The extract of the rhizome posses significant antibacterial activity with average zone of inhibition 11-19 mm. Maximum antibacterial activity property shown against Salmonela paratyphi, having the zone of inhibition of 19mm. Minimum antibacterial property shown against Bacillus subtilis, Sarcina luteae. Escharichia coli and Candida albicans, with the zone of inhibition of 11mm.

RESULT OF ANTIOXIDANT ACTIVITY TEST

% scavenging of the DPPH free radical was measured using the following equation-

Inhibition free radical DPPH in percent (1%) was calculated as follows:

$$(1\%-(1-A_{Sample/}\ A_{Blank}\ )\ X\ 100$$

Table 3: LC_{50} values of Ascorbic acid.

Sl. No.	Concentration (µg/ml)	Absorbance Of extract	Absorbance Of blank	% Inhibition	LC_{50} (µg/ml)
1.	500	0.053	0.42	85.75	22.784
2.	250	0.099	0.42	75.25	
3.	125	0.0154	0.42	61.5	
4.	62.5	0.166	0.42	58.5	
5.	31.25	0.179	0.42	55.25	
6.	15.625	0.198	0.42	50.5	
7.	7.8125	0.207	0.42	48.25	
8.	3.90625	0.213	0.42	46.75	
9.	1.953	0.227	0.42	43.25	
10.	0.97656	0.238	0.42	40.5	

Table 4: LC_{50} values of crude methanolic extract of Zingiber officinale rhizome.

SI No.	Concentration (ug/ml)	Absorbance of extract	Absorbance Of blank	% Inhibition	LC_{50} (ug/ml)
1.	500	0.102	0.325	68.61538	239.167
2.	250	0.103	0.325	68.30769	
3.	125	0.138	0.325	57.53846	
4.	62.5	0.248	0.325	23.69231	
5.	31.25	0.250	0.325	23.07692	
7.	7.813	0.263	0.325	19.07692	
8.	3.906	0.267	0.325	17.84615	
9.	1.953	0.269	0.325	17.23077	
10.	0.977	0.273	0.325	16	

Result and Discussion of Antioxidant Activity of DPPH

The methanol extract of rhizomes were subjected to free radical scavenging activity by the method of Brand-Williams et al. (1995). And ascorbic acid was used as standard in this investigation. The antioxidant activities of the leafy vegetables of Bangladesh were measured by free radical-scavnging assay with DPPH. LC_{50} values (concentration of sample required to scavenging 50% free radical to the activity.The extract was found to have different levels of antioxidant activity.

Table-4 shows that LC_{50} value of methanol extract of rhizomes of Zingiber officinale plants was 239.166 which indicating medium potential antioxidant properties.

CONCLUSION

The study was conducted to evaluate the anti-microbial activity and anti-oxidant activity of the whole plant. The highest zone of inhibition of this extract was 19 mm in diameter and it

was against *salmonella paratyphii*. In conclusion it is confirmed that the methanol extract of rhizome have moderate anti-oxidant activity.

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