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CONTENTS

Study of Zingiber Officinale and Evaluation in vitro Anti-Bacterial and Anti-Oxidant Activity

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Abstract: A root of *Zingiber officinale* extracts from Ethanol, Chloroform, Carbontetrachloride, Methanol, and Hexane were investigated for their in vitro antimicrobial properties, with disk-diffusion and minimal inhibition concentration (MIC) method. All extract of *Zingiber officinale (Bel)* were different in terms of their antibacterial activities. The ethanol act showed a stronger and broader spectrum of antibacterial activity, study was also carried out to evaluate the in-vitro antioxidant activities of ethanol ,chloroform and carbon tetrachloride extract of *Zingiber officinale* species namely. This was achieved by screening the two plant extracts at varying concentrations (10-50ìg/ml) using DPPH radical scavenging activity. The results were analyzed statistically which showed that ethanol extract *Zingiber officinale* has more antioxidant activity than standard antioxidant.
Key words – Antioxidant activity, Antibacterial activity, DPPH Test.

Introduction

Ginger is a tropical plant that was spread around the tropical world during the colonial days. In Africa, Asia, or South America, and many hot spots between those continents, locals have taken to using ginger widely in medicine and food. The entire ginger family is rich in oils that both kill micro-critters and stimulate the immune system to do the same. The ways it stimulates the body are many and whether added to food or taken as a medicinal tea [1-5], ginger makes your body a little bit stronger and a little bit better able to resist the damaging forces of nature. Here are the basics for using ginger as a medicine. Damage to cells caused by free radicals is believed to play a central role in the aging process and in disease progression. Antioxidants are our first line of defense against free radical damage and are critical for maintaining optimum health and wellbeing. The need for antioxidants becomes even more critical with increased exposure to free radicals [6-10]. Pollution, cigarette smoke, drugs, illness, stress, and even exercise can increase free radical exposure. Because so many factors can contribute to oxidative stress [11-16], individual assessment of susceptibility becomes important. Many experts believe that the Recommended Dietary Allowance (RDA) for specific antioxidants may be inadequate and, in some instances, the need may be several times the RDA [17-18].

Material and method

Plant Material:

The plant materials used in this study, *Zingiber officinale* roots, seed and leaves of were collected from the field in Latur District. A voucher specimen of the collected sample was deposited in our institutional herbarium for the reference. Preparation of various extract of *Zingiber officinale*.

In present study we use dry stem of the plant collected from western ghat region Maharashtra. Dried Fruit, Leaves and Seeds are cut into small pieces these pieces are then grinded. The grinded sample is dark or gray brown in colour with a special smell.This powder of all three separately stirred in non-polar solvent Carbon tetra chloride, for 1/2 hour & then it is refluxed for 1/2 hour this is performed for extraction of non-polar component from powder. After extraction the Carbon tetra chloride layer is distilled to recover solvent & to get a brown colored liquid fraction which shows single spot on thin layer chromatography.The residue of Carbon tetra chloride extraction is used for further study. This residue is mixed with Chloroform and stirred for 1/2 hour & then refluxed for 1 hour. After filtration the filtrate is distilled to get Chloroform Fraction which is Red-brown colored liquid.Then the Residue of Chloroform is used for extraction with Ethyl acetate stirred well & refluxed for 1 hour then filtered. Filtrate is then distilled & fraction of Ethyl acetate is collected it shows no spot on TLC plate. Conclusion is that no organic compound is present.The Ethyl acetate residue is further mixed with ethanol & stirred for 1/2 hr and refluxed for 1hr. Then it is filtered & filtrate is distilled out. Ethanol fraction is yellow brown in colour & show three spot on TLC plate .All extract Collected in Bottle and studied for various activities.

Antioxidant Activity

DPPH Scavenging Test:

Quantitative measurement of radical scavenging property was carried out in a universal bottle. The reaction mixture contained 50 µL of test samples (or 80% MeOH as blank) and 5 mL of a 0.004% (w/v) solution of DPPH in methanol. Different known antioxidants, vitamin E, and butylated hydroxytoluene (BHT, Sigma) were used for comparison or as a positive control. Discoloration was measured at 517 nm

after incubation for 30 min. Measurements was taken at least in triplicate. DPPH radical's concentration was calculated using the following equation: DPPH scavenging effect (%) = [(Ao – A1) / Ao] ×100; Where Ao was the absorbance of the control and A1 was the absorbance in the presence of the sample. The actual decrease in absorption induced by the test compounds was compared with the positive controls. The mean OD 517 results of DPPH scavenging activity were recorded.

Antimicrobial Activity:

The agar diffusion method [11] was used to evaluate the antimicrobial activity. Bacteria were cultured overnight at 37 ^{o} C in Mueller Hinton 10 µl Broth and fungi at 28 ^{o} C for 72h in Potato Dextrose Broth (PDB, Oxide) and used as inoculums. A final inoculums, using 100 µl of suspension containing 10 ^{8} CFV/ml of bacteria 10 ^{4} spore/ml of fungi spread on Mueller Hinton Agar (MHA) and Potato Dextrose Agar (PDA) medium respectively.

The disc (6 mm in diameter) was impregnated with 10 µl of 75 µl/ml, 50 µl/ml, 25 µl/ml, 10 µl/ml and 5 µl/ml of each extracts and for each organism placed on seeded agar. Ciprofloxacin and Fluconazole (75 µl/ml, 50 µl/ml, 25 µl/ml, 10 µl/ml and 5 µl/ml) were used as positive control bacteria and fungi respectively. The test plates were incubated at 37 ^{o} C for 24h for bacteria and at 28 ^{o} C for 72h for fungi depending on the incubation time required for a visible growth.

MIC values were also studied for microorganisms by turbid metric method, which were determined as sensitive to the extracts in cup plate method. MIC was defined as the lowest concentration of extract that inhibit visible growth.

Observation tables

Table-1 Antibacterial activity of Ethanolic extracts seeds Zingiber officinale

Bacterial	Extract Ethanolic	Extract CCl_4	Cefotax	Pencil	Tetrax
G (+)	In mm	In mm	In mm	In mm	In mm
1.Staphylococcus epidermidis	10	9.2	10	10	9
2. Staphylococcus aureus	08	10.6	10	8	8
3. bacillus paludisme	12	6.4	11	10	8
4. Bacillus subtilis	14	9.4	11	10	7
G (-)					
1. Escherichia coli	5	8.7	6	5.5	5.5
2. Pseudomonas aeruginosa	8	7.8	6	7	4.5
3. Bacillus subtilis	7	8.5	4.5	8.5	9
4. Enterobacter aerogenes	4	5.7	6	5	6

Table-II: Antifungal activity of Ethanolic extracts from extract from seeds Zingiber officinale

Fungus	**Extract Ethanoli**	**Extract CCl_4**	**Cefotax**	**Penicil**	**Tetrax**
1. Candida albicans	6.5	6.8	7	5	9
2.Aspergillus fumigatus	8.3	3.6	5	7	4
3. Aspergillus niger	4.7	10.5	10	8	9

Table-III :Antibacterial activity of methanolic extract from root of *Zingiber officinale*

Bacteria	Extract Ethanolic	Extract CCl_4	Cefotax	Penicil	Tetrax
G (+)	In mm	In mm			
1. Staphylococcus epidermidis	6.6	7.2	10	10	9
2. Staphylococcus aureus	8.8	7	10	8	8
3. bacillus paludisme	6.8	9.7	11	10	8

4. Bacillus subtilis	9.8	5.4	11	10	7
G (-)					
1. Escherichia coli			6	5.5	5.5
2. Pseudomonas aeruginosa	7.3	3.4	6	7	4.5
3. Bacillus subtilis	8.5	6.3	4.5	8.5	9
4. Enterobacter aerogenes	8.2	7.4	6	5	6
1. Staphylococcus epidermidis	8.3	4.3	10	10	9

Table-IV
Antifungal activity of Ethanolic extract from root of Zingiber officinale

Fungus	Extract ethanolic	Extract CCl_4	Cefotax	Penicil	Tetrax
1. Candida albicans	4.0	5	7	5	4
2.Aspergillus fumigatus	7.2	8	5	6	4
3. Aspergillus niger	4.2	8	6	4	5

Table-V: Antioxidant activity of Fruits

Extract Conc. Mg/ml	BHT	Ethanol	$CHCl_3$	CCl_4
0.05	45.1	20.60	21.53	17.47
0.1	46.91	36.64	14.53	10 .70
0.2	49.24	24.24	27.50	24 .50
0.3	57.57	40.12	44.00	30 .50

Table VI: Antioxidant activity of root

Extract Conc. Mg/ml	BHT	Ethanol	CHCl3	CCl4
0.05	45.1	18	15	17
0.1	46.91	21	23	25
0.2	49.24	23	27	29
0.3	57.57	45	30	267

Results And Discussion:

There is a strong need for effective antioxidants from natural sources as alternatives to synthetic antioxidant in order to prevent the free radicals implicated diseases which can have serious effects on the cardiovascular system, either through lipid per oxidation or vasoconstriction The extracts and essential oils of many plants have been investigated for their antioxidant activity [5-7]. Secondary metabolites such as polyphenols are not required for plant development and growth, but are involved in plant communication and defense [8-9]. Polyphenols interact with pathogens, herbivores, and other plants; they protect from ultraviolet radiation and oxidants, repel or poison predators and attract beneficial insects or microbes [10-11]. Therefore, in this study, the antioxidant properties of the methanol extracts of leaves and stems of plant like of reexamined for DPPH radical scavenging activity according to the method described and the results of the screening are shown in table 1 & table 2 as comparable with known antioxidant BHT. In terms of antioxidant activity, all the extracts investigated exhibited a rather high degree of activity (more than 40%). In particular, fruits (ethanol extract) of *Zingiber officinale* displayed the highest activities as antioxidant activity as removal of the stable radical DPPH and the lowest activity were found in CCl4 *extract of stem.* As expected, the overall activity of the raw extracts was lower than that of commercial antioxidant BHT, the reference antioxidant. The results of Antimicrobial activity were done for all the five, pet ether, chloroform, acetone, and methanol and aqueous extracts. During antimicrobial study methanolic extracts showed maximum zone of inhibition against almost all organisms in cup plate methodThe ethanolic extract from roots of *Zingiber officinale*showed a good inhibition against all the bacterial Strains tested (MIC

between 10&80 ug/ml). The gram (+) bacteria were sensitive with gram (-) bacteria and some common fungi.

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