Quantitative Estimation of Bioactive Compound Present in Gingiber Officinale Extract and Its Antioxidant and Antimicrobial Activity

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Abstract— Ginger (Zingiber officinale) has long been used as natural medicine due to their potential antimicrobial activity against different microbial pathogens. Ginger is one of the important medical plants which naturally occurs in various countries like India, China. SouthEast Asia, West Indies, Mexico and some other countries of world also. Zingiber officinale belongs to family zingiberaceae. This is a type of storage root having pungent taste. This study was conducted to assess the antioxidant property and antimicrobial susceptibility of ginger against some bacterias. The antimicrobial sensitivity of bacteria is carried out by disk diffusion method at different concentration. Two bacterial isolates that is E.coli and pseudomonas putida were tested at different concentration of ginger extract that is 25, 50, 75 and 100%. The findings has shown that E.coli was most affected by the ginger extract followed by pseudomonas putida and the bacterial growth inhibition is higher at 75% concentration. The medicinal properties of ginger are due to the presence of gingerol and paradol, shogaols, etc. Medicinal value of ginger and its knowledge provide the good platform for researchers for future research to protect human beings from several types of diseases.

Key words: Ginger (Zingiber officinale)

I. INTRODUCTION

The importance of medicinal plants in solving the health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats. The worldwide spread use of herbal remedies is not restricted to developing countries, as it has been estimated that 70% of all medical doctors in France and Germany regularly prescribe herbal medicine (Murray et al 2000). Most of the developing countries have adopted traditional medical practice as an integral part of their culture. The number of patients treated by herbal approaches for therapy is also growing exponentially. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc. Recent estimates suggest that several thousands of plants have been known with medicinal applications in various cultures. Plants constitute an important source of natural products which differ widely in their structures, biological properties and mechanism of action. Various phytochemical components especially polyphenols, flavonoids, phenolic acids etc. are responsible for the free radical scavenging and antioxidant activity of the plants.

Fig. 1: Ginger plant

Fig. 2: Rhizosome of Ginger

Ginger (Zingiber officinale Roscoe) is a in the flowering plant family Zingiberaceae whose rhizome, ginger root or simply ginger, is widely used as a spice or a medicine. It is an herbaceous perennial which grows annual stems about a meter tall bearing narrow green leaves and yellow flowers. Ginger has a number of regional uses. It is widely used in cooking and as a main ingredient in traditional ayurvedic medicines.

Ginger extract and its pungent compounds demonstrated greater antibacterial activity against a variety of bacteria species including H. pylori, Staphylococcus aureus, Pseudomonas aeruginosa and E. coli.

From 1585, Jamaican ginger was the first oriental spice to be grown in the New World and imported back to Europe. In 2012, India, with over 33% of the global production, now leads in growing ginger, replacing China, now in second position (about 20%), followed by Nepal (about 12%), Nigeria and Thailand (each about 7%) and Indonesia (about 5%).

A. Medicinal Use and Research

According to the American Cancer Society, ginger has been promoted as a cancer treatment "to keep tumors from developing," but "available scientific evidence does not support this." They add: "Recent preliminary results in animals show some effect in slowing or preventing tumor growth. While these results are not well understood, they deserve further study. Still, it is too early in the research process to say whether ginger will have the same effect in humans." 30In limited studies, ginger was found to be more effective than placebo for treating nausea caused by seasickness, morning sickness, and chemotherapy, although it was not found superior to placebo for pre-emptively treating postoperative nausea. Some studies advise against taking ginger during pregnancy, suggesting that ginger is mutagenic, though some other studies have reported antimutagenic effects.

B. Phytochemistry of Zingiber Officinale

Phytochemicals which not only that they are nutritive plants chemicals they have protective or disease preventive properties but also protect human from a host of disease. Phytochemical studies have shown that plants with antimicrobial activity contain bioactive constituents such as tannins, flavoinoids, alkaloidsa and saponnins. Alkaloids and flavonoids have been used as antiviral, antibacterial and anticancer agents. Phenolic and polyphenolic are the other group of secondary metabolites. The uses of plant – derived products are disease control agents have been studied, since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance.

II. MATERIAL AND METHODS

A. Collection of plant sample and processing

The Plant materials of Zingiber Officinale was collected from the area near S block, Nehru nagar, Bhopal. The root of Gingiber officinale was grind by Mechanical technique to obtain fine powder.

1) Extraction

The root material fine powder in a grinding machine. A quantity of 20 g of the dried powder of Gingiber officinale root extracted with Methanol (Merck) using Soxhlet. The soxhletion with analysis, Extract was evaporated in water bath at Methanol solvent were due for a 4-5 days to obtained extract. After that, the Determination of Bioactive compound and 50ºC to obtained crude for antioxidant assay, Phytochemical and Antimicrobial Susceptibility (Gupta et al., 2011)

2) Phytochemical Analysis

Phytochemical analysis is a major procedure for estimation of particular chemical compound. Phytochemical analysis was preceeded by the help of different specific method for each test. All phytochemical analysis (test) was done by the using Kokate (Practical (Khandelwal 2008) Pharmacognosy) as a reference book. The phytochemical test which was performed is mentioned in below:

Test for carbohydrates and proteins, Test for tannins and saponnins, Test for alkaloids and flavonoids, Test for glycosides and steroids, Test for terpenoids.

3) Determination of Total Phenolic Content (TPC)

Extract in the concentration of 1mg/ml was used in the analysis. A dilution series of tannic acid (3mg/3ml) (standard) prepared at different concentrations. Blank was alongside prepared 1 ml Phenol Folin-ciocalteu's reagent dissolved in water and 1 ml Na2CO3. 2 ml of sample solution was also made with concentration 600µl sample, Phenol Folin-ciocalteu's reagent, Na2CO3. The samples were thereafter incubated for 90 min. The absorbance was determined using spectrophotometer at λmax = 760 nm

4) Determination of Total Flavomoid Content (TFC)

Flavonoids content of isolated crud [Root powder (Gingiber officinale)] were determined this method (Shin J et al., 1999). Take a clean test tube and add 0.5 ml of the sample (Extract) containing 1.25 ml of distilled water. Then added 3mg/3ml of Ascrbic acid solution and allowed to stand for 5 min. Added 0.15 ml of 10 % aluminium chloride, after 6 min 0.5 ml of 1.0 M sodium hydroxide were added and the mixture were diluted with another 0.275 ml of distilled water. The absorbance of the mixture at 510 nm was measured immediately

5) Thin Layer Chromatography (TLC)

Approximately 10 ml of crude extract is dissolved in methanol. Which acts as stationary phase. Silica plates were made by spreading the slurry of silica gel, prepared by mixing silica gel and d/w uniformly in mortar – pestle. A uniform layer is applied on clean glass slide with spatula, and then dried at room temp. Followed by drying at 60^0 C in hot air oven. Solvent system was prepared Ethyl acetate (9.1): Methanol (2.8), which acts as mobile phase. The spot of methanol extract and water extract was applied on plates using capillary tube then; the plates are kept straight in solvent system prepared in beaker that contains developing solvent up to depth of about 0.5cm for ascending developing of thin layer chromatogram, the glass jar was covered with petriplates to provide saturated atmosphere. The solvent was allowed to run on plates till solvent reaches the highest point of plates.

6) Antioxidant Activity

To calculate anti-oxidant 15ml Solution (reaction mix.) was made with H2SO4, Na2HPO4 and (NH4)6MO7024.H2O (Prieto et al., 1999). 2 different dilution was made 1 with standard (Ascorbic Acid) with different conc. (300µl, 600µl, 900µl, 1200µl, 1500µl) and other with sample of different conc. (1ml, 1.5ml, 2ml, 2.5ml, 3ml). A blank was prepared with H2SO4, Na2HPO4 and (NH4)6MO7024.H2O, Test tubes were then incubated at 90ºc for 90 min.Then after cooling the absorbance was taken at 695nm in spectrophotometer against blank.

7) Nitric Oxide Scavenging Activity

The procedure is based on the principle that, sodium nitropruside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Gris reagent. Scavengers of nitric oxide complete with oxygen, leading to reduced production of nitrite ions. Plant extract was dissolved in distilled water for this quantification. Sodium Nitropruside (5mM) in standard phosphate buffer saline (0.025m, pH 7.4) was incubated with different concentration (100-400µg/ml) of methanol extract and tubes were incubated at 29ºC for 3 hours. Control experiment

without the test compounds but with equivalent amount of buffer was conducted in an identical manner. After 3 hours incubated samples were diluted with 1 ml of Gris reagents. The absorbance of the color developed during diazotization of Nitrite with sulphanilamide and its subsequent coupling with Napthylethylenediaminehydro-chloride was observed at 550nm on spectrophotometer. Same procedure was done with ascorbic acid which was standard in comparison to methanol extract. Calculated the % inhibition by formula and plot graph in compared to standard (Singh et al., 2012).

$$\text{Nitrous Oxide Scavenged (\%)} = \frac{A_{control} - A_{Test}}{A_{control}} \times 100$$

Where,

$A_{Control}$ = Absorbance of control reaction.

A_{test} = Absorbance in the presence of the samples of extract.

8) Antimicrobial Activity

80 ml NAM was prepared (peptone- 0.4g, beaf extract- 0.2g, NaCl- 0.4g, Agar- 1.2g, d/w- 80 ml),and autoclaved for 20 min. The crude extract was dissolved in distilled water and four concentrations of the extract were made (100%, 75%, 50%, and 25%). The media was poured in petriplates in laminar and left to solidify. After solidify of media in petriplates, the given bacteria (Ecoli, & Pseudomonas spp) was gently spread on the media and four columns were made to test the antimicrobial activity at different concentration of extract. The whatman paper discs (6mm diameter) were dipped in the all four concentrations of sample and put in the media. It was then incubated for 24 hours. After overnight inoculation of culture media. Zone of inhibition was observed.

III. RESULTS

A. *Phytochemical Investigation*

S.No.	Phytochemical test	Observation
1.	Carbohydrate Molish test Cobalt chloride	 +ve +ve
2.	Protein Million test Biuret test Xanthoprotein test	 +ve -ve +ve
3.	Steroids Salkowiski reaction	 +ve
4.	Glycosidase Killer-killiani test	 -ve
5.	Anthroquinone glycosidase Borntragers test	 -ve
6.	Flavonoid Flavonoid test	 +ve
7.	Alkaloids Mayers test Wagner test	 -ve +ve
8.	Tannic & phenolic compound 5% $Fecl_3$ sol^n Lead acetate sol^n Gelatin sol^n Acetic acid sol^n Dil. Iodine sol^n	 +ve +ve -ve +ve -ve
9.	Organic test Confirmatory test for malic acid	+ve
10.	Inorganic acid Sulphate test	 +ve
11.	Chloride Chloride test	 +ve

Table 1: Phytochemical Investigation

Fig. 3: Phytochemical Analysis

B. *Total Phenolic Content*

Total phenolic contents of Zingiber officinale was found to be 122.75µg/ml with response to standard curve of tannic acid.

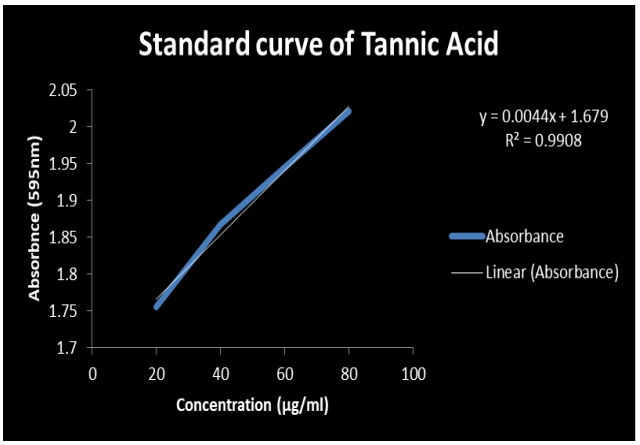
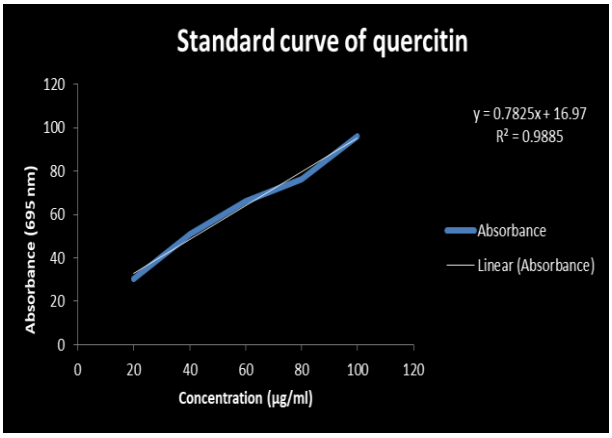


Fig. 4

C. *Total Flavonoid Content*

Total Flavonoid contents of Zingiber Officinale was found to be 83.94µl with response to standard curve of Quercetin.



Fig, 5

D. Total Antioxidant Scavenging Activity

Using of scavenging %. Plot standard curve graph of Ascorbic acid. Absorbance of standard and sample at different concentration at 695nm

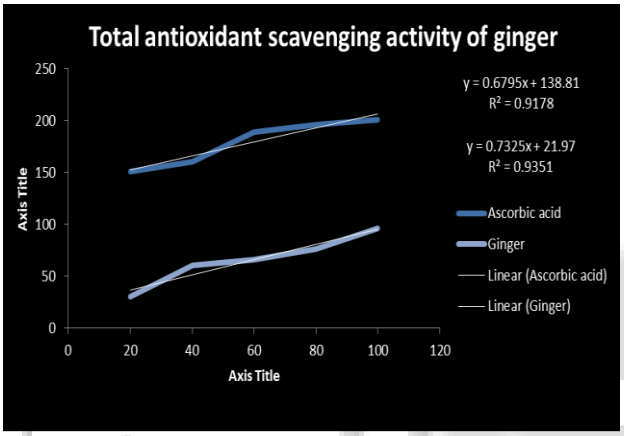


Fig. 6: Standard curve of Ascorbic acid at different concentration.

E. Nitrous Oxide Scavenging Activity

Using of scavenging %. Plot standard curve graph of Ascorbic acid. Absorbance of standard and sample at different concentration at 550nm

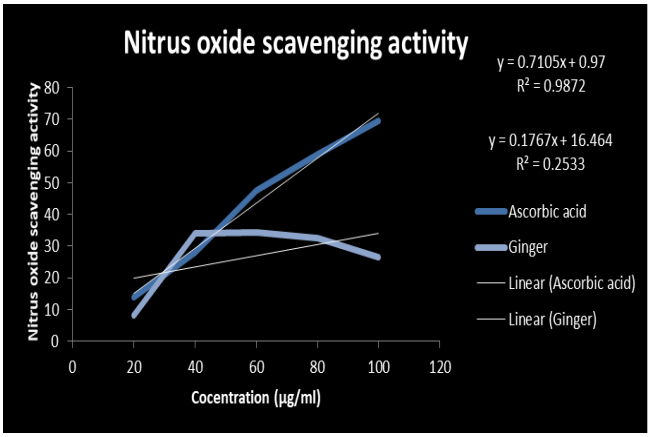


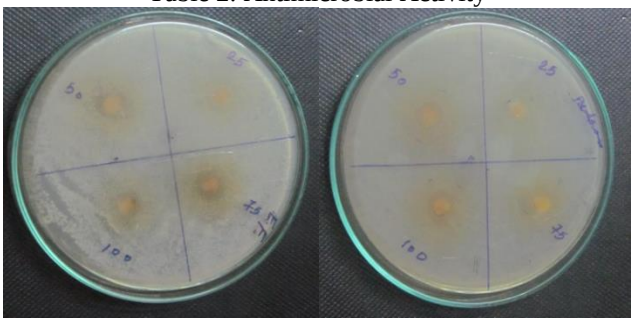
Fig. 7: Standard curve of Ascorbic acid at different concentration

F. Antimicrobial Activity

Zone of inhibition by Ecoli. & Pseudomonas putida different concentration

Concentration(300mg/3ml) zone of inhibition(mm)		
	E.coli	Pseudomonas putida
25%	13mm	10mm
50%	15mm	11mm
75%	17mm	12mm
100%	14mm	10mm

Table 2: Antimicrobial Activity



E.coli Pseudomonas putida

Fig 8: Zone of inhibition around disc of different concentration

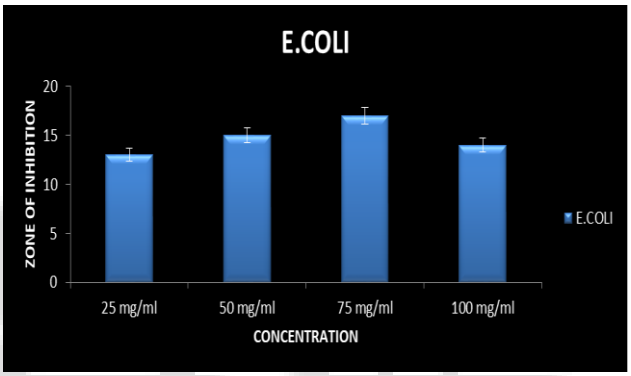


Fig. 9: Zone of Inhibitioon of E.coli

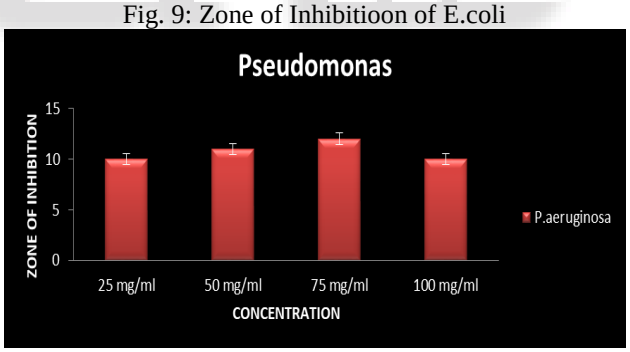


Fig. 10 Zone of Inhibition of Pseudomonas

G. Thin Layer Chromatography

Qualitative analysis done by TLC using chloroform (7.2) and ethanol (2.8) solvent system.

Fig. 11: TLC of Zingiber officinale (in UV)
After observation the band showing Flavonoid Content.

IV. CONCLUSION

Based on the result in the study, it was concluded that extracts of Zingiber Officinale Roscoe were found to be a good natural antioxidant. Like for the antimicrobial activity, the antibacterial activity may be attributed, possibly in combination, to various phytochemicals detected during the extracts chemical screening and which are known to cause damage to cell membranes, causing leakage of cellular materials and ultimately microorganisms' death. Further studies are required to identify specific active principles of this plant for the significant antioxidant effect.

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