

ANTIMICROBIAL AND ANTIFUNGAL ACTIVITIES OF GREEN CHILI, GREEN PAPAYA, GREEN AND RIPE TOMATOES AND BITTER GOURD

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ABSTRACT

The antibacterial activity of Green Papaya, Green Tomato, Ripe Tomato, Green Chili and Bitter Gourd was screened in vitro against 5 gram positive (*Bacillus megaterium*, *Bacillus subtilis*, *Bacillus cereus*, *Sarcina lutea*, *Staphylococcus aureus*) and 5 gram negative strains (*Salmonella typhi*, *Salmonella paratyphi A*, *Shigella dysenteriae*, *Shigella sonnei*, *Escherichia coli*) of pathogenic bacteria. Disc diffusion technique was used for the antibacterial screening. Growth of the gram positive strains was inhibited by all of the samples. Gram negative strains of bacteria were found to have limited sensitivity against samples. The antifungal activity of the five samples were evaluated in vitro against 5 fungi (*Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus asper*, *Aspergillus ustus*, *Aspergillus ochraceus*). Only Green Chili gave zone of inhibition against *Aspergillus ochraceus*.

KEYWORDS: Antibacterial, Antifungal, Disk diffusion technique, Green Papaya, Green Tomato, Ripe Tomato, Green Chili, Bitter Gourd.

INTRODUCTION

Food has long been represented the sustenance of man and the only means of treatment available against illness.^[1] Depending on the concentration of the active principles, they are

considered as drugs or food. Green Chili contains bioactive compound capsaicin which is a safe and effective topical analgesic agent in the management of arthritis pain, herpes zoster-related pain, diabetic neuropathy, post mastectomy pain, and headaches.^[2] Green papaya is a rich source of anti-oxidants, fiber, enzymes, vitamins and minerals. Its composition makes it a potent weapon against serious diseases like cancer and heart disease, and helps to maintain the healthy functioning of the digestive organs. Green tomato contains three times more calcium than red tomato. They also contain slightly more vitamin C than red tomato. Red tomato, in comparison, has more folate and potassium than green tomato. It is rich in lycopene, one of the most powerful natural antioxidants, has been found to help prevent prostate cancer. Green unripe fruit of the tomato plant contains small amounts of the poisonous tomatine but ripe tomatoes do not contain tomatine. Bitter gourd or bitter melon is largely found in several Asian countries. It is rich source of vitamin C and has blood sugar lowering effect.^[4] It possesses various chemicals such as charantin, peptides resembling insulin and several alkaloids. Due to these constituents, it is quiet widely used in the treatment of diabetes mellitus. Vegetables are particularly important sources of micronutrients and vitamins, e.g., provitamin A, B6, C and E as well as folic acid.^[5]

There are five main mechanisms by which antibacterial agent act^[6]: by inhibiting the metabolism of a microorganism, but not the metabolism of the host; by inhibiting the synthesis of bacterial cell wall that leads to bacterial cell lysis and death; by interacting with the plasma membrane cells of bacteria to affect membrane permeability that has fetal results for the bacterial cell; by disrupting the protein synthesis of the bacterial cell so that it can no longer survive; by inhibiting nucleic acid transcription and replication of bacterial cell. Antifungal works by exploiting differences between mammalian and fungal cells to kill the fungal organism without dangerous effects on the host.

MATERIALS AND METHODS

Collection of the sample

5kg of each sample Green Chili, Green Papaya, Green Tomato, Ripe Tomato and Bitter Gourd were collected from Mirpur-1 kacha bazar.

Preparation of extract

The fresh Green Papaya, Green and Ripe Tomato, Green Chili, Bitter Gourd were washed with water immediately after collection. They were chopped into small pieces, sun dried for about 10 days and ground into powder form and stored in a container. Two hundred gm

powder was macerated in 200ml methanol for 7 days at room temperature with occasional stirring. After that, methanol extract was filtered off through a cotton plug and finally with a Whatman No.1 filter paper. The extracts were concentrated and allowed to air dry for complete evaporation of methanol. The whole process was repeated four times and finally 16.6gm of Green Chili, 41.71gm of Green Papaya, 40.73gm of Green Tomato, 32.03gm of Ripe Tomato and 15.21gm of Bitter Gourd sample extract were obtained which was kept in a refrigerator at 4°C.

Screening of antibacterial activities

In this work the antibacterial activity of the Green Papaya, Green and Ripe Tomato, Green Chili, Bitter Gourd was determined by “Disk diffusion method”.^[7] It is essentially a quantitative or qualitative test indicating the sensitivity or resistance of the microorganisms to the test materials. However, no distinction between bacteriostatic or bacteriocidal activity can be made by this method.

Green papaya, Green and Ripe tomato, Green chili, Bitter gourd were tested in vitro for their antibacterial activity against a number of clinical isolates of gram positive and gram negative bacterial strains. Identified pure isolates of all the gram positive strains were obtained from the Microbiological Laboratory of the Institute of Nutrition and Food science, University of Dhaka, and that of the gram negative strains were supplied by the Pathological Laboratory of the ICDDR'B (International Centre for Diarrhoeal Disease and Research, Bangladesh), Dhaka. Gram positive bacteria used for the study were *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus cereus*, *Sarcina lutea*, *Staphylococcus aureus* and the Gram negative strains were *Shigella sonnei*, *Escherichia coli*, *Salmonella typhi*, *Salmonella paratyphi A*, *Shigella dysenteriae*.

The test organisms were transferred to the agar slants from the supplied pure cultures with the help of an inoculating loop in aseptic condition. Special care was taken to avoid contamination of microorganism. The inoculated slants were then incubated at 37°C for 24 hours to assure the growth of test organisms.

The media used for the study were Nutrient agar medium and Nutrient broth medium. Nutrient agar culture medium was composed of 0.5g of bacto peptone, 0.5g of sodium chloride, 1.0g of bacto yeast extract, 2.0g of bacto agar, 100ml of distilled water and its pH was 7.2±0.1 at 25°C. Nutrient broth medium was composed of 1.0g of bacto yeast extract,

0.5g of sodium chloride, 0.5g of bacto peptone, 100ml of distilled water and its pH was 7.2 ± 0.1 at 25°C .

After preparation of the media, they were autoclaved and then cooled to 45°C . Petri-dishes and paper discs were sterilized in the hot air oven at 180°C for 2 hrs, cool in normal temperature with covering. Micropipette tips, cotton, forceps, blank discs etc were also sterilized.

Green Chili, Green Papaya, Green Tomato, Ripe Tomato and Bitter Gourd extracts were taken to other vials by weighing about 0.5g and were labeled with 1, 2, 3, 4, 5 respectively. Methanol and chloroform (500 μl) were added to each vial which was then shaken gently. Sterilized filter paper discs (4mm in diameter) were taken in a blank Petri-dish. Sample solution of desired volume (30 μl) was applied on each disc with the help of a micropipette in an aseptic condition for complete removal of solvent. Then they were dried in room temperature.

About 10ml of the medium was poured carefully in each Petri dish of 90mm in diameter. The Petri-dishes were rotated several times first clockwise and then anticlockwise and allowed to cool at room temperature and then stored in refrigerator at 4°C . In the disc diffusion assay, the surface of a nutrient agar medium contained in a Petri dish was uniformly inoculated with the test bacterial subculture. The filter paper discs applied with test sample solution were placed on each of the Petri-dishes that were previously inoculated.

The plates were kept in an incubator at 37°C for 24hrs to allow maximum growth of the organisms. If the test material have any antibacterial activity, it will inhibit the growth of bacteria giving a clear distinct zone called “zone of inhibition” around the test material contained in the filter paper discs. The antibacterial activity of the test samples were determined by measuring zone of inhibition in millimeter. Each experiment is repeated twice or thrice. This method was developed by Bauer *et. al* in 1966 for susceptibility test.

Screening of antifungal activity

Antifungal activities of different sample extracts were tested against five fungi using disc diffusion technique, as it is essentially a quantitative or semi quantitative test indicating the sensitivity or resistance of microorganism to the test material.

The cultures of the fungal strains were taken from the Microbiological Laboratory of the Institute of Nutrition and Food science, University of Dhaka. The fungal strains used in the sensitivity test are: *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus asper*, *Aspergillus ustus*, *Aspergillus ochraceus*.

Potato Dextrose Agar (PDA) media was used to perform the antifungal activity test and for subculture of the test organisms. The media was composed of 20gm of peeled and sliced potato, 2gm of dextrose, 2gm of agar, and 100ml of distilled water.

Green Chili, Green Papaya, Green Tomato, Ripe Tomato and Bitter Gourd extracts were taken to other vials by weighing about 0.5g and were labeled with 1, 2, 3, 4, 5 respectively. Methanol and chloroform (500µl) were added to the each vial and shake gently.

Antifungal discs were prepared for antifungal activity screening. Sterilized filter paper discs (4mm in diameter) were taken in a blank Petri-dish. Sample solution of desired volume (30µl) was applied on each disc with the help of a micropipette in an aseptic condition. The discs were left for a few minutes in the aseptic condition for complete removal of solvent. Before preparation of the test plates a number of Petri-dishes, a piece of cotton, the medium were sterilized by autoclave and then transferred to the laminar air flow cabinet.

About 10ml of the medium was poured carefully in each medium sized petridishes. The Petri-dishes were rotated several times first clockwise and then anticlockwise to assure homogenous thickness of the medium and allowed to cool at room temperature and solidify at about 30°C.

In the disc diffusion assay, the surface of a potato dextrose agar medium contained in a Petri dish was uniformly inoculated with the test fungal culture. Test sample solution was applied on filter paper disc with the help of a micropipette and dried in room temperature. The filter paper discs were then placed on each of the Petri-dishes that were previously inoculated by means of sterile forceps. The discs were placed in such a way that was not closer than 15mm to the edge of the plate apart from each other to prevent overlapping of the zone of inhibition. The plates were kept in a refrigerator into the medium. Finally the plates were incubated at 37°C for 24hrs in an incubator. If the test material have any antifungal activity, it will inhibit the growth of fungus giving a clear distinct zone called “zone of inhibition” around the test material contained in the filter paper discs.

The antifungal activity of the test samples were determined by measuring zone of inhibition in millimeter.

RESULTS AND DISCUSSION

Of the 10 bacterial strains tested, gram positive strains were observed to have prominent sensitivity towards the samples studied. Among gram negative strains *Salmonella typhi*, *Salmonella paratyphi A*, *Shigella dysenteriae* were ineffective to all of the samples. Green Chili and Bitter Gourd each gave a zone against *Shigella sonnei* and Green Tomato against *Escherichia coli*. All of the samples did not give zone against others gram negative bacterial strains.

Only Green Chili was effective against *Aspergillus ochraceus*. Other samples were not showed anti fungal activity against *Aspergillus fumigatus*, *Aspergillus nigar*, *Aspergillus asper*, *Aspergillus ustus*, *Aspergillus ochraceus*.

Table1. Antibacterial activity of the samples against pathogenic bacteria (inhibitory zone in mm)

Test Bacteria	Strain No.	Inhibitory Zone in mm				
		Sample				
		Green Chili	Green Papaya	Green Tomato	Ripe Tomato	Bitter Gourd
<i>Shigella dysenteriae</i>	T1683	—	—	—	—	—
<i>Bacillus megaterium</i>	QL32	10mm	23mm	22mm	28mm	32mm
<i>Bacillus subtilis</i>	QL40	—	8mm	—	—	9mm
<i>Bacillus cereus</i>	QL29	6mm	—	7mm	7mm	—
<i>Sarcina lutea</i>	QL166	7mm	11mm	8mm	14mm	21mm
<i>Staphylococcus aureus</i>	QL102	—	15mm	17mm	13mm	—
<i>Shigella sonnei</i>	A116958	6mm	—	—	—	20mm
<i>Escherichia coli</i>	CRL	—	—	18mm	—	—
<i>Salmonella typhi</i>	QL147	—	—	—	—	—
<i>Salmonella paratyphi A</i>	CRL	—	—	—	—	—

‘—’ indicate no inhibitory zone

Antibacterial activity of the vegetable extract is due to the presence of alkaloids, glycosides, flavonoids and phenolic compounds which have been shown to posses antibacterial and antifungal properties.^[7] So in this study, we have conducted various in vitro experiments to assess the antibacterial and antifungal effect of Green Chili, Green Papaya, Green Tomato, Ripe Tomato and Bitter Gourd sample extract against pathogenic bacteria and fungi.

In the present study, disc diffusion method has been used for screening of antibacterial activity. On the basis of result obtained in the present investigation, Green Chili, Green Papaya, Green Tomato, Ripe Tomato and Bitter Gourd sample extract have considerably significant in vitro antibacterial activity.

Among the tested bacteria, Gram positive strains *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus cereus*, *Sarcina lutea*, *Staphylococcus aureus* were observed to have prominent sensitivity towards all the samples. All the samples were found to have strongest activity against *Bacillus megaterium*. Green Papaya and Bitter Gourd are effective against *Bacillus subtilis* and Green Chili, Green and Ripe Tomato sample against *Bacillus cereus*. All the samples have antibacterial activity against *Sarcina lutea*. Green Papaya, Ripe and Green Tomato are effective against *Staphylococcus aureus*. Effective samples give zone of inhibition in mm.

On the contrary, Gram negative strains of bacteria have limited sensitivity nearly to every samples. Green Chili and Bitter Gourd show antibacterial activity against *Shigella sonnei*. Green Tomato is effective against *Escherichia coli*. Others gram negative bacteria *Salmonella typhi*, *Salmonella paratyphi A*, *Shigella dysenteriae* are almost resistant to all of the samples and sample extracts have no effect against those bacteria.

The reason for the highest effectiveness of samples on the above gram positive strains is not clear. Among all of the samples, Green Tomato and Bitter Gourd are most active to inhibit the growth of bacteria. Other samples also have antibacterial activity but they are not so much active as sample Green Tomato and Bitter Gourd.

Screening of antifungal activity is also done by the disc diffusion method. Five fungi *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus asper*, *Aspergillus ustus*, *Aspergillus ochraceus* were used tested the vegetable extract. The fungi were observed to be insensitive to the extract, except the green chilli against *Aspergillus ochraceus*. It gave 10mm zone of inhibition.

CONCLUSION

In summary, these fruits and vegetables appear to have important medicinal properties which make it therapeutically useful in first line treatment of various diseases. Add these vegetables in our diet may have preventive effect to many fatal diseases. Due to these pharmacological

activities, we assume that different active secondary metabolites are present and perhaps some of the compounds may operate in a synergistic manner. The results of the investigation do not reveal that which chemical compound is responsible for mentioned activity. However, further study is required to confirm its potentiality and prospect in pharmacological effects. This research statement may serve as a preliminary step on this aspect. Now our study will be directed to explore the specific compound responsible for previously mentioned activity and extensive in vitro and clinical research is required to explore its medicinal values.

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