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Green bell pepper to treat UTI, oral disease, intestinal infection through its phytochemical agent

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Abstract
The main reason for this study is to investigate the antibacterial activities of green bell pepper (*Capsicum annum*) against some common bacteria. Here three types of bacteria are used for this study such as *E. coli, S. mutant, P. vulgaris*. In this study, green bell pepper was used for its antibacterial activity against *E. coli* isolated from humans with Diarrhea, *S. mutant* isolated from human tooth decay, oral cavity, or inflammation on the buccal surface, and *P. vulgaris* isolated from the intestinal tract of human.

Keywords: reason, antimicrobial, isolated, inflammation

Introduction
Capsicum annum is also known as "Green Bell Pepper". It is an automatic vegetable that is used as food, have a special spicy flavour. We take many antibiotics against many different diseases, but they have many side effects. Many pathogenic bacteria can also resistance this type of antibiotic. So many medicinal plants are used to resist them. There have no side effects. Green bell pepper is also used in the medicinal field. Green bell pepper is an excellent source of many phenolic compounds, bioactive compounds, antioxidants, free radicals, and vitamins. Green bell pepper consists of many antioxidant compounds such as Carotenoids, Vitamin C, Capsaicinoids, Fatty acids (linoleic acid, palmitic acid, and alpha-linolenic acid), Tocopherols, especially phenolic compounds (such as flavonoids and phenolic acids). Many free radicals belong to human etiology including cancer, cardiovascular disease, diabetes, etc. These phenolic compounds and other essential compounds may inhibit the growth of some microorganisms. Green bell pepper has some effective activity against *E. coli*, *S. mutant*, and *P. vulgaris*. *Escherichia coli* is a Gram-negative bacteria generally found lower intestine and also in the gut of some animals. Most types of *E. coli* are helped in our digestive system but some strains can cause Diarrhea if we take contaminated food or water. It also can cause urinary tract infections. *E. coli* produce Shiga toxin that makes us very sick. *Streptococcus mutant* is a Gram-positive bacteria mainly found in our buccal surface or oral cavity. It is a causative agent of our tooth decay. *S. mutant* causes an oral cavity in children. *Proteus vulgaris* is a Gram-negative bacterium that is found in our intestinal tract and also the fecal matter of animals. It can also occur some diseases like wound infection and urinary tract infection. So now we studied the antibacterial activities of *Capsicum annum* extract against some bacteria that are already discussed before in the text.

Materials and Methods
Site of Experiment
The total experiment was carried out in the laboratory room of Rabindra Mahavidyalaya, Champadanga, Hooghly, West Bengal, India.

Collection of Microorganisms
The tested microorganisms (*E. coli, Streptococcus mutant, and Proteus vulgaris*) brought together from MTCC Chandigarh, India. The microorganism's strains remained in Mueller Hinton Agar (MHA, pH -7.2) at 37 degrees Celsius. The stock culture remained at 4 degrees Celsius.

Collection of Plant Material
The testing vegetable (*Capsicum annum*) was collected from the nearby market of Champadanga, Hooghly, W. B., India.


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Fig 1 and 2: *Capsicum annum*

Taxonomic Position
Super kingdom: Eukaryota
Kingdom: Viridiplantae
Phylum: Streptophyta
Order: Solanales
Family: Solanaceae
Subfamily: Solanoideae
Tribe: Capsiceae
Genus: Capsicum

Fig 3: Fruit extract (dust)

Preparation of Fruit Extract
At first, the collected vegetables were washed under the running tap water for 2-3 times. Then they are finally washed with distilled water and remain under shade for a few days at room temperature. Then the dried materials converted to dust by a mixer

Different Extraction
Methanol Extraction
To prepare methanol extraction, at first we take 5gm of dried powder of fruit extract of *Capsicum annum* in a 100ml conical flask and added 20ml of methanol and shake them very carefully. Then they were covered with a tissue paper tightly with a rubber band. At last, some small pores created above the tissue paper for passing air and evaporation. They transferred at room temperature for 48 hours.

Fig 4: Methanol extraction

Ethanol Extraction
To prepare ethanol extraction, at first we take 5gm of dried powder of fruit extract of *Capsicum annum* in a 100ml conical flask and added 20ml of ethanol and shake them very carefully. Then they were covered with a tissue paper tightly with a rubber band. At last, some small pores created above the tissue paper for passing air and evaporation. They transferred at room temperature for 48 hours.

Acetone Extraction
To prepare acetone extraction, at first we take 5gm powder of fruit extract of *Capsicum annum* in a 100ml conical flask and added 20ml of acetone and shake them very carefully. Then they were covered with a tissue paper tightly with a rubber band. At last, some small pores created above the tissue paper for passing air and evaporation. They transferred at room temperature for 48 hours.

Benzene Extraction
To prepare benzene extraction, at first we take 5gm of dried powder of fruit extract of *Capsicum annum* in a 100ml conical flask and added 20ml of benzene and shake them very carefully. Then they were covered with a tissue paper tightly with a rubber band. At last, some small pores created above the tissue paper for passing air and evaporation. They transferred at room temperature for 48 hours.

Preparation of extract concentration
We prepare four extracts before. From these four extractions *viz*. Methanol, Ethanol, Acetone and Benzene, using DMSO we prepared four concentrations such as 50µg/ml, 100µg/ml, 200µg/ml, and 400µg/ml.

Collection of Antibiotics
The antibiotics (Ampicillin) were collected from the nearby market of Champadanga, Hooghly, West Bengal, India.

Microbiological Assay
Agar Disc Diffusion Method
The antibacterial screening of fruit extract of *Capsicum annum* was prepared by dissolving 4gm of each extract separately in 10ml Dimethyl Sulphoxide (DMSO). The prepared diluted extract was used for microbial study. Sterile Agar Medium was bored to create a suitable wall in the Petri plate by using a borer. Only two wells were created on a Petri plate, one of these remains for control (DMSO), and the other well is used for antibiotics of similar concentration.

Medium
At first, we take 100ml of water then added 3.8gm of Mueller Hinton Agar (MHA). They shake very well. Then it autoclaved at 1210C for 15 minutes at fifteen lbs and transferred to a Petri plate, which is already sterile. The agar pours homogeneously thickness about 4 millimetres. Then the agar remains at a low temperature until it became cool.

Inoculums and Incubation
In the agar plates, 0.1ml of microorganism's cultures was transferred.
The inoculated plates were remained for 5 minutes before creating wells in the agar for different concentrations to be tested.
Then the extracts of *Capsicum annum* were poured at different concentrations in the well on the agar plate. Then the plates were allowed to incubate at 37 degrees Celsius for 24-48 hours in a laboratory incubator.

Statistical Analysis
After incubation, we see a clear zone around the well in the plate that is called the "zone of inhibition". We measured the zone of inhibition using a zone reader. We take three nearly similar values of the zone of inhibition for each concentration of *Capsicum annum* extract. The obtained

values were analyzed using SIGMAPLOT software (version – 14.0). The resulting data were analyzed by using "t-test".

Phytochemical Estimation

Extract Preparation

20gm of dry powder was added in Methanol, Ethanol, Acetone, and Benzene in a conical flask. They are shaken carefully in a rotator shaker for 24 hours. Then we collected the supernatant and the solvent was evaporated.

Phytochemical Studies

The Phytochemical study is a qualitative study. Phytochemical constituents are the medicinal value that lies in these plants, leaves, fruits, or the part which tested that produces physiological action on the human body. The phytochemicals have a great role in the pharmacognostic drug development and treatment of many ailments. There are found man phytochemicals such as steroids, alkaloids, tannins, flavonoids, reducing sugar, and different phenols.

Test for Steroids

When we tested for steroids, we take 2ml of the test solution and mixed it with 2ml of Acetic Anhydride followed by 2ml of Sulphuric Acid.

Then we see the color changed from violet to blue or green in some samples that indicate the steroids present in the solution.

Test for Flavonoids:

We take 2ml of the test solution which mixed with 4ml of 1% Aluminium Chloride in Methanol in a test tube. Then we observed that yellow color formed, indicated the presence of flavonoids.

Test for Alkaloids

Alkaloids are nitrogenous compounds which have much physiological and pharmacological activity. We detect the presence of Alkaloids by Mayer's reagent test. A few drops of Mayer's reagent added in the alkaloids solution that produced white-yellowish precipitate. The fruit extract was stirred with 5ml of 1% HCl on a steam bath. Then it filtered. Add 2 drops of Mayer's reagent in the 1ml filtrate and observe the precipitation and we know that alkaloids are present.

Test for Tannins

At first 1 gm of fruit extract powder was taken into a beaker and 10ml of distilled water add and the mixture boiled for 5 minutes. Then 2 drops of 5% FeCl3 were added. A blue-green color arises that indicates the presence of Tannins.

Test for Reducing Sugar

In 0.5 ml of extract solution, we added 1ml of water and 5-8 drops of Fehling's solution and hit them a few minutes. Then we observed a brick-red precipitate and sure that reducing sugar is present.

Result

We used both polar and non-polar solvents for the extraction of active components from the fruit of green bell pepper fruit. The antibacterial activities were determined using the agar disc diffusion method by measuring the diameter of the growth inhibition zone.

Table 1: Phytochemical analysis of secondary metabolites such as alkaloids, flavonoids, steroids, tannin, reducing sugar present in *Capsicum annum.*

Phytochemicals compounds	Green bell pepper fruit
Alkaloids	+++
Flavonoids	++
Steroids	-
Tannin	-
Reducing sugar	-

Table 2: The antibacterial activity of Methanol extract of *Capsicum annum* against various bacteria.

Concentration (µg/ml)	Zone of inhibition (mm)		
	Escherichia coli	***Streptococcus mutant***	***Proteus vulgaris***
400 µg/ml	36.5±1.0	38.0±2.0	32.8±2.0
200 µg/ml	32.2±2.0	33.2±1.0	30.0±1.0
100 µg/ml	28.6±1.0	29.0±2.0	27.1±1.0
50 µg/ml	25.2±0.0	25.7±0.0	22.2±2.0

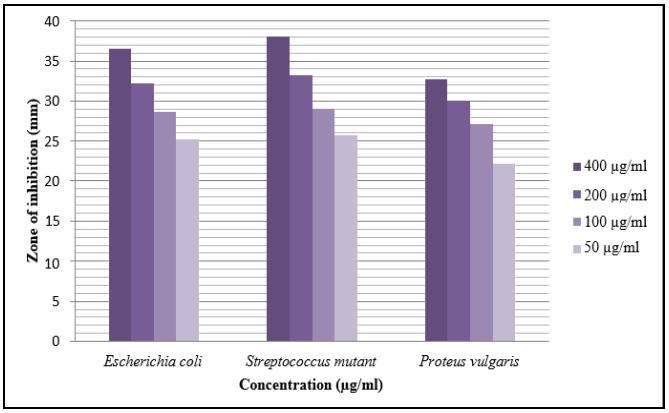

Fig 5: The antibacterial activity of Methanol extract of *Capsicum annum* against various bacteria.

Table 3: The antibacterial activity of Ethanol extract of *Capsicum annum* against various bacteria.

Concentration (µg/ml)	Zone of inhibition (mm)		
	Escherichia coli	***Streptococcus mutant***	***Proteus vulgaris***
400 µg/ml	37.0±1.0	40.0±2.0	34.4±2.0
200 µg/ml	33.0±2.0	36.2±1.0	31.2±1.0
100 µg/ml	28.8±2.0	29.1±2.0	28.0±1.0
50 µg/ml	25.8±1.0	26.2±0.0	23.6±2.0

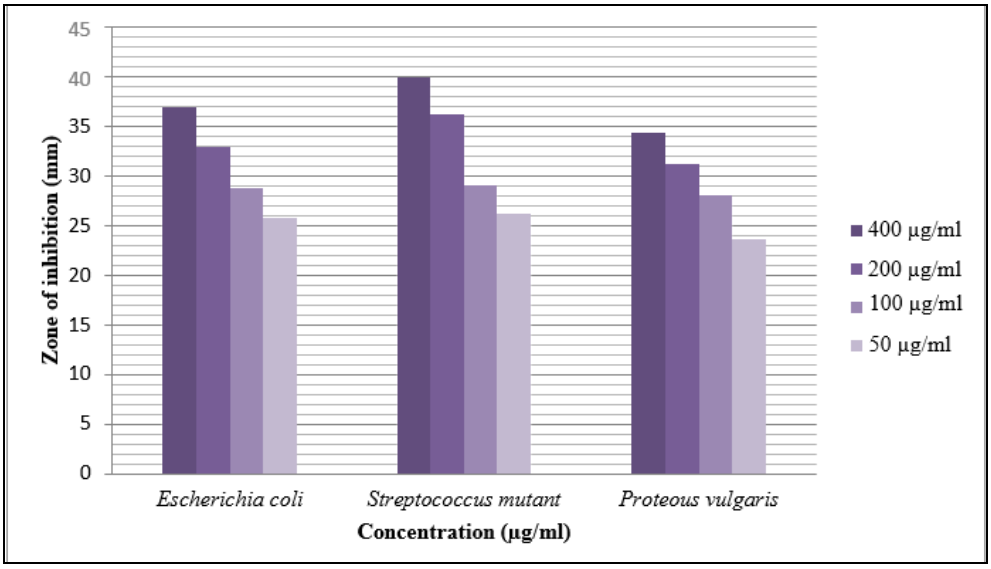

Fig 6: The antibacterial activity of Ethanol extract of *Capsicum annum* against various bacteria

Table 4: The antibacterial activity of Acetone extract of *Capsicum annum* against various bacteria

Concentration (µg/ml)	Zone of inhibition (mm)		
	Escherichia coli	***Streptococcus mutant***	***Proteus vulgaris***
400 µg/ml	32.0±1.0	36.0±2.0	30.0±2.0
200 µg/ml	28.2±2.0	30.2±1.0	26.4±1.0
100 µg/ml	25.4±2.0	27.0±2.0	24.1±0.0
50 µg/ml	23.0±3.0	24.2±0.0	21.0±2.0

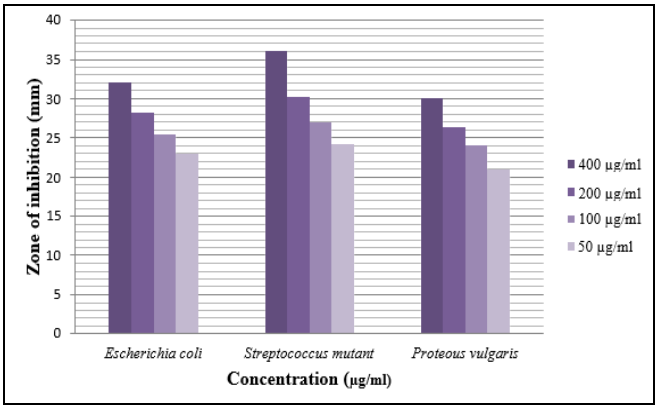

Fig 7: The antibacterial activity of Acetone extract of *Capsicum annum* against various bacteria

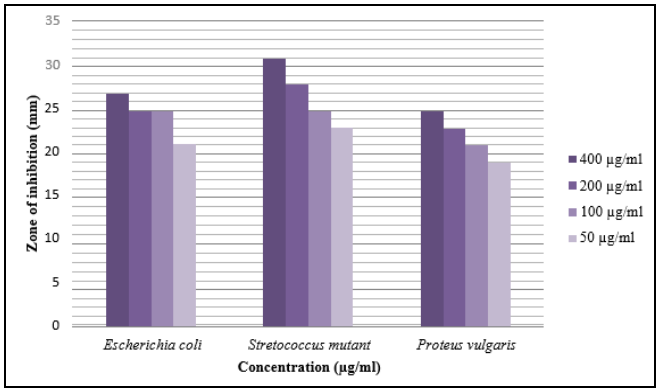

Fig 8: The antibacterial activity of Benzene extract of *Capsicum annum* against various bacteria.

Table 5: The antibacterial activity of Benzene extract of *Capsicum annum* against various bacteria

Concentration (µg/ml)	Zone of inhibition (mm)		
	Escherichia coli	***Streptococcus mutant***	***Proteus vulgaris***
400 µg/ml	26.8±1.0	30.6±2.0	25.0±2.0
200 µg/ml	25.0±0.0	28.1±0.0	23.4±1.0
100 µg/ml	24.6±2.0	25.2±2.0	20.8±1.0
50 µg/ml	21.4±3.0	23.4±1.0	19.2±2.0

Conclusion

Many plants, their fruits, and their seeds have much medicinal values that help our health and other purposes. The study on green bell pepper fruit extract has much antibacterial activities on different bacteria. The antibiotics treated to resist many bacteria, ampicillin, the plant extract as the same concentrations inhibit many bacteria. So the fruit extract is used in many medicinal and pharmaceutical fields. The fruit extract is easy to prepare. So hopefully we use the

green bell pepper extract as a medicinal field. Therefore, green bell pepper could be effectively used as a natural spicy food in our everyday meal, for the prevention of bacterial infection. Indeed this phenomenal fruit may serve as a good resource for the spicy industry and clinical medicine. This research could be further extended to test the bioactive properties of green bell pepper for therapeutic uses.

Future Aspect

There were wide futures aspects of Research work with Green bell pepper. Green bell pepper should certainly find a place in the various treatment of bacterial infection. The results from the present study we concluded that it is very useful in the treatment of bacterial infection. It was discovered that the phytochemicals present in the green bell pepper extract inhibited the growth of microorganisms and the result compared with antibiotics, Ampicillin commonly used in therapeutics and they showed less strong inhibition for Gram-negative bacteria and more strong inhibitory effect for Gram-positive bacteria. *Capsicum annum* shows significant activity as because the fruit contains many useful phytochemical compounds which have much medicinal values in the treatment of diarrhea, pneumonia, urinary tract infection, wound infection, tooth decay, etc.

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