



EVALUATION OF ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF PAPAYA AND PINEAPPLE FRUIT JUICES AGAINST SELECTED ENTERIC PATHOGENS

BANSODE D.S.* AND CHAVAN M.D.

Department of Microbiology, Walchand College of Arts and Science, Solapur (M.S.)-413006-India.

ABSTRACT

In recent years multiple resistances in human pathogenic microorganisms have developed due to indiscriminate use of antibiotic drugs commonly employed in the treatment of infectious diseases. The objective of present study was to evaluate antimicrobial activity and phytochemical analysis of fruit juices of Papaya (*Carica papaya*) and Pineapple (*Ananas comosus*) against medically important selected enteric pathogens. The Papaya and pineapple fruit juices were subjected to screening against enteric pathogens *E.coli*, *Salmonella paratyphy B*, and *Shigella sonnei*. Antimicrobial analysis was done by using the agar well diffusion method against selected enteric bacteria. The MIC values were determined by using U.V. Spectrophotometer. The fresh crude Pineapple fruit juice produced the highest antimicrobial activity against *E.coli* followed by *Shigella sonnei* and *Salmonella para.B*. and fresh crude Papaya fruit juice produced the highest antimicrobial activity against *Shigella sonnei* followed by *E.coli* and *Salmonella para.B*. The phytochemical analysis showed presence of protein, carbohydrate, flavonoids, glycosides, steroids and reducing sugar. The antimicrobial activity of standard antibiotic Ampicillin was studied in comparison with papaya and pineapple fruit juices. The Ayurvedic system of medicine has described various fruit juices in the treatment of diseases, which play an important role in modern health care and curing various ailments and diseases. The uses of herbal medicines are increasing as a dietary supplement to fight or prevent common diseases. It has great demand for primary health care because plant based medicines; health product, pharmaceuticals, food supplements, cosmetics etc. are thought to be non toxic, have less side effects and easily available at affordable cost. There are several reports on antimicrobial activity of crude fruit juices that inhibits various bacteria. However limited number of antimicrobial activity of *in vitro* studies on various fruit juices has been published, it has not been determined whether they are superior or equivalent to antibiotics. Therefore scientific evaluation of these fruit juices is the need of hour to check their antimicrobial activity and supports their use as an alternative medicine in the treatment of enteric infections. Hence an attempt was made, to screen the antimicrobial analysis and phytochemical study of fresh fruit juices of Papaya and Pineapple in the prevention of enteric bacterial infections.

KEYWORDS: Antimicrobial analysis, Papaya and Pineapple fruit juices, enteric pathogens, MIC, phytochemical study.



BANSODE D.S.

Department of Microbiology, Walchand College of Arts and Science, Solapur (M.S.)-413006-India.

*Corresponding author

INTRODUCTION

The increase in antibiotic resistant bacteria is largely due to the widespread use of antibiotics in medicine, in animal care, and in agriculture. The problem is compounded by the lack of new antibiotics to attack bacteria in different ways to circumvent the resistant genes. Decreasing efficiency and resistance of pathogens to antimicrobial drugs made the search of a new antimicrobial agent an important strategy for the establishment of alternative therapies in difficult handling infections. *E.coli*, *Salmonella paratyphi B*, and *Shigella sonnei* are about the major causes of intestinal infections. Enteric bacteria belong to the family Enterobacteriaceae, which are a large group of Gram negative, peritrichously flagellated or non-flagellated straight rods with simple nutritional requirements. They grow best under aerobic condition but also ferment carbohydrates by an anaerobic pathway. Enterobacteria include coliforms like *E. coli* and other Gram-negative enteric flora, slow lactose fermenting bacteria which could be either normal flora or regular pathogens also form part of the enteric pathogens. Enteric Pathogenic bacteria have developed resistance against existing antibiotics due to indiscriminate use of antimicrobial drugs to treat the infectious diseases. The worldwide emergence of *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and many others β -lactamase producers has become a major therapeutic problem⁴. The fruit juices exhibit significant antibacterial effect, the bioactivity being associated with mineral content and biologically active constituents. Hence these fruit juices with the property of bioavailability and retention of certain minerals by polyphenolic compounds can be recommended for their use as an alternative anti-infective agent in natural medicine for the treatment of infectious diseases⁶. The herbal products have less side effects and easily available at affordable cost⁹. Gastrointestinal diseases are the most frequent causes of morbidity and mortality in developing countries. The presence of enterobacteria in foodstuffs and water is a

common cause of diarrhea and dysentery among the infant population. *Escherichia coli* is a classic example of enteric bacteria capable of producing diseases⁷. Recent years, papain and other endopeptidases have been proven to have several medical benefits, such as defibrinating wounds and treatment of edemas⁵. Papaya contains some specific antimicrobial substrates including Carpaine and Aglycons. Papaya is nutritious fruit full of medicinal properties in both its ripe or raw form. All the fruits are good for human health. But amongst all papaya tops the rank. Papaya is rich source of multiple nutrients like carotenes, vitamin C, flavonoids, and fiber. It is also an excellent source of vitamin B, folate and pantothenic acid as well as minerals like potassium manganese and iron. The Pineapple fruit is an important food which can be eaten fresh or eaten in a processed form. It is composed of nutrients which are good for human health. This is due to researches carried out on the relationship between nutrients in pineapple and human health. Pineapple is largely consumed around the world as canned pineapple slices, fruit salads, sugar syrup, alcohol, citric acid, pineapple chips and pineapple puree. In the tropics, pineapple is grown and used as a medicinal plant. Pineapple contains the enzyme Bromelain (Protease) which has several therapeutic properties including malignant cell growth, thrombus formation, inflammation, control of diarrhea, dermatological and skin debridement. The available evidence indicates Bromelain is well absorbed orally with its therapeutic effects being enhanced in a dose dependent manner and if successfully incorporated in feeds, it could become more acceptable as a nutraceutical product.

MATERIALS AND METHODS

1. Preparation of Fresh Pineapple and Papaya Fruit Juices

The fresh Pineapple and Papaya fruits were purchased from the local market of Solapur (MS). The procured fruits were washed and dried

at room temperature. The outer hard portion of these fruits was removed by using knife and fresh juice was collected by using a sterile syringe for further activity.

2. Bacterial strains and cultural conditions

Three strains of enteric pathogenic bacterial cultures were used in this study. These are *E.coli*, *Salmonella paratyphy B*, and *Shigella sonnei*. The pure pathogenic bacterial strains were collected from Dept.of Microbiology, Dr.V.M. Government Medical College, Solapur (MS). The collected pure pathogenic bacterial strains were isolated from hospitalized patients at Govt. Civil Hospital, Solapur (MS). The cultures were maintained on nutrient agar slants at 4°C and subcultures for 24hrs before use.

3. Evaluation of Antimicrobial Activity

The antimicrobial analysis method was performed to evaluate the antimicrobial properties of fresh Pineapple and Papaya Juices with the help of the agar well diffusion method. Nutrient agar plates were prepared to evaluate the Antimicrobial Activity of fresh Papaya and Pineapple juices against selected enteric pathogens. 50ul inoculums of each selected bacterium was uniformly spread on nutrient agar plates with the help of glass spreader, after five minutes 6mm diameter well was bored in the plates with the help of sterile cork borer. 50ul of fresh juice samples and standard antibiotic ampicillin were poured into the well with the help of sterile syringe. The plates were allowed to diffuse in a refrigerator for about 30min and then transferred to bacterial incubator. The plates were incubated at 37°C for 24hr. and after incubation plates were observed for the zone of inhibition.

4. Determination of the Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial that inhibits or kills the visible growth of microorganisms. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against

microorganisms. Four different concentrations i.e. 100%, 75%, 50%, 25% of these juices were prepared by diluting with distilled water. The nutrient broth was prepared. From this broth 9ml broth was added in each of four test tubes labeled as 100%, 75%, 50%, 25% fruit juices concentrations. Three sets of four test tube containing 9ml nutrient broth were prepared for three test microorganisms. Then 1ml of each concentration of fruit juices was added into the respective test tube. After this step 0.1ml test pathogen suspension were inoculated into respective labeled test tube. After inoculation, the test tubes were kept in a shaker incubator for overnight at 37°C and results were observed in the form of turbidity and O.D.were observed at 600nm on U.V.Spectrophotometer.

5. Phytochemical Analysis

Qualitative phytochemical analysis

The fruit juices were tested for the presence of bioactive compounds by using following standard methods³.

5.1 Test for proteins

Ninhydrin test

Crude juice when boiled with 2ml of 0.2% solution of Ninhydrin, violet Colour appeared suggesting the presence of amino acids and proteins.

5.2 Test for carbohydrates

Iodine test

Crude juice was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

5.3 Test for phenols and tannins

Crude juice was mixed with 2ml of 2% solution of FeCl₃. Blue-green or black coloration indicated the presence of phenols and tannins.

5.4 Test for flavonoids

Alkaline reagent test

Crude juice was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of

few drops of diluted acid which indicated the presence of flavonoids.

5.5 Test for saponins

Crude juice was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

5.6 Test for glycosides

Salkowski's test

Crude juice was mixed with 2ml of chloroform. Then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown color indicated the presence of steroidal ring, i.e. glycone portion of the glycoside.

5.7 Test for steroid

Crude juice was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2ml of chloroform. Then 2ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

5.8 Test for reducing sugar

Juice was shaken with distilled water and filtered. Filtrate was boiled with Fehling's solution A&B for 10 min. Red precipitate indicates the presence of reducing sugar.

RESULTS

Fruit juices are valuable and readily available resources for primary health care and complementary health care system, undoubtedly fruit juices containing substances of medicinal value that have yet to be discovered, though large number of fruit juices are constantly being screened for their antimicrobial effect, these fruits may prove to be a rich source of compounds with possible antimicrobial activities. In the present study agar well diffusion method was used here in order to get

antimicrobial activity of fresh Papaya and Pineapple juice against enteric pathogens. MIC was also done to know the minimum inhibitory concentration of fruit juices by using U.V.Spectrophotometer. The antimicrobial activity of fresh Papaya and Pineapple juices were represented in table 1-4. The zone of inhibition against various selected enteric pathogens were measured in mm. Zone of inhibition were seen against all species, confirming the antimicrobial activity of fresh Papaya and Pineapple juices. Table no.2 and 4 represented the antimicrobial activity of various concentrations of Papaya and Pineapple fruit juices of conc. 100%, 75%, 50%, 25%. In the present study, the zone of inhibition observed to all of the conc. of fresh Papaya and Pineapple juices including 25% concentration. This suggests that the minimum conc. of Papaya and Pineapple juices shows antimicrobial activity against selected enteric pathogens. The antimicrobial activity of standard antibiotic Ampicillin was represented in Table no.1 and 3 in comparison with Papaya and Pineapple juices. The antimicrobial activity of standard antibiotic Ampicillin was found to be maximum against *Sal.Para.B* (8mm) followed by *Shigella sonnei* (6mm) and *E.coli* (4mm). The fresh crude Pineapple fruit juice produced the highest antimicrobial activity against *E.coli* (6mm) followed by *Shigella sonnei* (4mm) and *Salmonella para.B* (4mm). and fresh crude Papaya fruit juice produced the highest antimicrobial activity against *Shigella sonnei* (4mm) followed by *E.coli* (3mm) and *Salmonella para.B* (3mm). Table no.2 and 4 represented the antimicrobial activity of various concentrations of Papaya and Pineapple fruit juices of conc. 100%, 75%, 50%, 25%. The fresh Papaya and Pineapple juices were subjected to get the MIC against selected enteric pathogens. The MIC observed at 75% conc. of pineapple juice against *E.coli*, 25% conc. of pineapple juice against *Sal.Para.B* and 50% conc. of pineapple juice against *Shigella sonnei*. The MIC observed at 50% conc. of papaya juice against *E.coli*, 75% conc. of papaya juice against *Sal.Para. B* and also 75% conc. of papaya juice against *Shigella*

sonnei. The phytochemical analysis showed presence of protein, carbohydrate, flavonoids, glycosides, steroids and reducing sugar. Thus results obtained in this study suggest the identified phytochemical compounds may be the bioactive constituents and these fruit juices can be used as potential source of drugs against intestinal diseases.

DISCUSSION

The present study suggested that, Papaya and Pineapple fruit juices have great potential as antimicrobial agents against selected enteric pathogens and they can be used as an alternative medicine in the treatment or control of enteric bacterial infections. This study supports the use of these fresh fruit juices of Pineapple and Papaya not only as dietary supplements but also as the agent to prevent or control the enteric bacterial infections. The result of antimicrobial susceptibility assay and the MIC assays showed promising evidence for the antimicrobial activity of fresh Papaya and Pineapple juices against enteric pathogens. Phytochemical analysis

showed presence of antimicrobial substances in fruit juices. Several studies have described the antioxidant properties of medicinal fruit juices which are rich in phenolic compounds². Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc.¹. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against a wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall. Steroids have been reported to have antibacterial properties⁸. Thus these fruit juices can be used as a potential source of drugs against enteric pathogens. The results revealed the presence of medicinally important constituents in the fruits juices. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Therefore, these fruit juices could be seen as a good source for useful drugs.

Table 1
Antibacterial activity of Pineapple Juice (Crude 100%).

Pathogens	Pineapple Juice (ZOI in mm)	Ampicillin (ZOI in mm)
<i>Shigella sonnei</i>	4	6
<i>E. coli</i>	6	4
<i>Sal.Para.B</i>	4	8

Table 2
Antibacterial activity of various conc. of Pineapple Juice.

Pathogens	Conc.of Pineapple Juice (%)	ZOI (mm)
<i>Shigella sonnei</i>	100%	4
<i>Shigella sonnei</i>	75%	3
<i>Shigella sonnei</i>	50%	2
<i>Shigella sonnei</i>	25%	1
<i>E. coli</i>	100%	6
<i>E. coli</i>	75%	4
<i>E. coli</i>	50%	2
<i>E. coli</i>	25%	2
<i>Sal.Para.B</i>	100%	4
<i>Sal.Para.B</i>	75%	3
<i>Sal.Para.B</i>	50%	2
<i>Sal.Para.B</i>	25%	1

Table 3
Antibacterial activity of Papaya Juice (Crude 100%).

Pathogens	Papaya Juice (ZOI in mm)	Ampicillin (ZOI in mm)
<i>Shigella sonnei</i>	4	6
<i>E. coli</i>	3	4
<i>Sal.Para.B</i>	3	8

Table 4
Antibacterial activity of various conc. of Papaya Juice.

Pathogens	Conc.of Papaya Juice (%)	ZOI(mm)
<i>Shigella sonnei</i>	100%	4
<i>Shigella sonnei</i>	75%	3
<i>Shigella sonnei</i>	50%	2
<i>Shigella sonnei</i>	25%	2
<i>E. coli</i>	100%	3
<i>E. coli</i>	75%	3
<i>E. coli</i>	50%	2
<i>E. coli</i>	25%	2
<i>Sal.Para.B</i>	100%	3
<i>Sal.Para.B</i>	75%	2
<i>Sal.Para.B</i>	50%	1
<i>Sal.Para.B</i>	25%	1

Table 5
MIC of various conc. of Pineapple Juice in nutrient broth, O.D. measured by U.V. Spectrophotometer.

Test Tube	Conc. of Pineapple Juice (%)	Pineapple Juice against <i>E. coli</i> (OD.at 600nm)	Juice against <i>Sal.Para.B</i> (OD.at 600nm)	Pineapple Juice against <i>Shigella sonnei</i> (OD.at 600nm)
1	100%	1.0467	1.1498	1.2231
2	75%	0.0017	1.0974	1.2245
3	50%	0.0019	1.3343	1.1362
4	25%	1.1251	1.0914	1.2864

Table 6
MIC of various conc. of Papaya Juice in nutrient broth, O.D. measured by U.V. Spectrophotometer.

Test Tube	Conc. of Papaya Juice (%)	Papaya Juice against <i>E. coli</i> (OD.at 600nm)	Papaya Juice against <i>Sal.Para.B</i> (OD.at 600nm)	Papaya Juice against <i>Shigella sonnei</i> (OD.at 600nm)
1	100%	1.4844	1.2806	1.4322
2	75%	1.5325	1.2243	1.1984
3	50%	1.3040	1.3907	1.5687
4	25%	2.1271	1.4042	1.6538

Table 7
Phytochemical analysis.

Test	Papaya Juices	Pineapple Juices
Proteins	+	+
Carbohydrate	+	+
Phenols / Tannins	+	+
Flavonoides	+	+
Saponines	+	-
Glycosides	+	+
Steroid	+	+
Reducing Sugar	+	+

+ = Present - = Absent



Figure 1: Antibacterial activity of Std. Ampicillin Against Enteric Pathogens

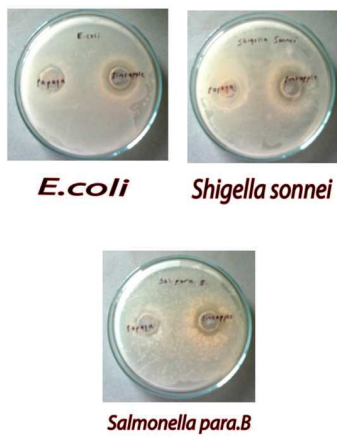


Figure 2: Antibacterial activity of Crude Papaya & Pineapple Juices

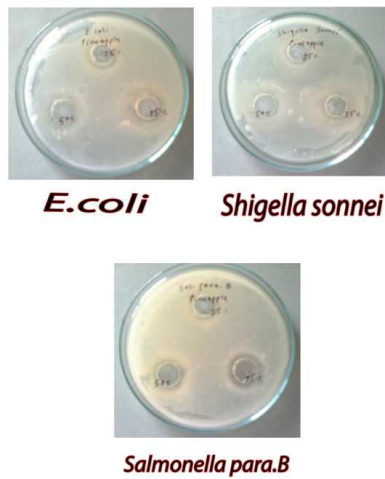


Figure 3: Antibacterial activity of various conc. Of Pineapple Juices

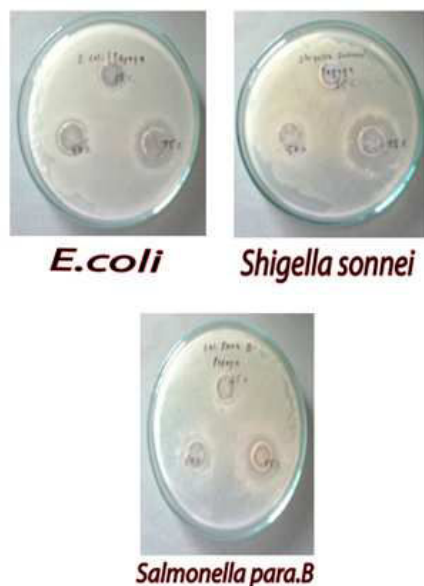


Figure 4: Antibacterial activity of various conc. Of Papaya Juices

CONCLUSION

The present study suggested that, the Pineapple and Papaya fruit juices have great potential as antimicrobial agents against selected enteric pathogens and they can be used as an alternative medicine in the treatment or control of enteric bacterial infections. The results of antimicrobial activity and MIC assays showed promising evidence for the antimicrobial activity of fresh Pineapple and Papaya fruit juices

against enteric pathogens. Phytochemical analysis showed presence of antimicrobial substances in the studied fruit juices. The results revealed the presence of medicinally important constituents in these fruit juices. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Therefore, the Pineapple and Papaya fruit juices could be seen as a good source for useful drugs.

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