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Phytochemical Analysis and Antibacterial activity of *Musa acuminata and Carica papaya* Peels against Food Poisoning Bacteria

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ABSTRACT

Food poisoning is a foodborne illness which causes due to the consumption of contaminated, spoiled, or toxic food products. Food poisoning is very common and frequently happens with people due to their unhealthy eating behavior. It comes with symptoms like abdominal cramps, vomiting, mild fever, weakness, stomach aches, etc. Fruits are one of the healthiest diets whenever taken, they always keep us healthy. But, apart from fruits, their peels also contain properties that are very beneficial for humans like antioxidants, antimicrobial, anti-inflammatory, fibrous, etc. Consuming raw peels isn't a good idea, but if extracted and incorporated in any product without affecting its property is considerable. This research is done to know the antibacterial property of *Musa acuminata and Carica papaya* peels. Phytochemical analysis of samples was done by ethanol extraction and shown that peel sample contains saponin, tannin, flavonoids, alkaloids, quinone, terpenoids which are antibacterial compounds. Isolation and identification of bacteria were done to isolate the food poisoning bacteria. The bacteria suspected were *Campylobacter jejuni* and *Bacillus cereus* after morphological and biochemical tests. Antibacterial screening of extracted samples was done by the disc diffusion method. *Carica papaya* has shown a 15mm and *Musa acuminata* 19mm inhibition zone against *Campylobacter jejuni, whereas Carica papaya* has shown 20mm and *Musa acuminata* 7mm inhibition zone against *Bacillus cereus.* A middling zone has shown that both the peel samples have an action against food poisoning bacteria and eligible to cure such diseases caused due to the bacteria when involved in any food product or nutraceutical.

Key words: Food poisoning, antibacterial, phytochemicals, Ethanol extraction, *Carica papaya, Musa acuminata, Campylobacter jejuni, Bacillus cereus,* Antibacterial screening, Zone of Inhibition.

INTRODUCTION

In the present environment, polluting substances are increasing day by day and causing adverse effects to the human body. These life-threatening pollutants are poisons, toxic gases, bacterial toxins, etc. which can be found in different areas and further became the reason for poisoning or foodborne diseases when involved in the food chain and hence, negatively affects the human body. The most common pathogens responsible for poisoning include bacteria (66%), chemical substances (26%), viruses (4%), and parasites (4%). Contamination to food can also happen due to microbes in animals, plants, sewage, soil, air, or improper handling of food which directly or indirectly enter the food and make the food unhygienic. (Hezam et al., 2019) According to the CDC, there are around 1, 28,000 people who are hospitalized and around 3,000 people who die of the foodborne disease each year. Very common symptoms of foodborne illness are abdominal pain, diarrhea, fever, dehydration ranging from mild, severe to death. (FSEP, 2016)

Food poisoning is also known as a foodborne illness caused due to consumption of contaminated food. Food poisoning happens because of the microorganisms like bacteria, viruses due to the toxins produced by them. Other than this, consumption of too many drugs, chemicals can also lead to poisoning when consumed in large amounts and they itself called poisons. Food poisoning illness is a global issue that affects people for a long. Some of the common food products like eggs, meat, raw milk, raw vegetables, are associated with food poisoning illness. One can notice the symptoms easily like dizziness, stomach pain, headache, vomiting, and diarrhea are major signs of food poisoning. These symptoms became life-threatening when they last for more than 3-4 days. (Aliyah, 2016) The common bacteria responsible for food poisoning are *Bacillus cereus, Campylobacter jejuni, Clostridium perfringens, Clostridium botulinum (very rare), Salmonella, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli* (Microbiology society) Food poisoning pathogens can be worst if not controlled appropriately since they grow in food, multiply and further affect the body, their level of affecting anybody is dependent on the individual's immune system so it is required to consume a hygienic and healthy diet.

Food poisoning is considered one of the most common outbreaks reported in 2017. 312 of the 1,649 outbreaks were the patient for acute diarrheal disease whereas 242 were reported as the patient for food poisoning which is a matter of concern as it will keep on increasing if proper measures were not taken. (Yasmeen ,2018). Foodborne or food poisoning cases are expected to rise by 177 million in 2030 which means by 2030, one out nine people on average might fall sick. (Whitworth, 2018). Food poisoning can happen to any group but there are certain groups of people who are more likely to affect by this illness because their bodies might not get the ability to fight against the pathogenic bacteria. These groups of people mostly include Children under 5 years old, Weak Immune systems, Pregnant women, Adults aged 65 or above.

Prevention is always better than cure, so it's good to avoid the things which cause food poisoning rather than treating it after. Below preventive measures would be useful if followed carefully like washing hands before and after eating food. Raw foods should be washed properly before eating; Cook foods thoroughly at the proper temperature, clean dishes and utensils should be used. Do not place cooked food on an unhygienic utensil., Avoid cross-contamination of foods like separate knives and cutting boards should be used for meat, eggs, and vegetables, Refrigerate perishable foods or leftover foods within 2 hours and avoid eating a long time unrefrigerated foods, Do not drink water from streams or wells that are not properly treated. Only drink water that is chlorinated or well-maintained etc. (Sowjanyaand Aliyah, 2016).Fruits and vegetables are a majorly utilized source out of all horticultural crops, consumption of which are either raw form, minimally processed form, or fully processed form. They can provide nutrients in the body as people are constantly changing their diet habit and heading towards the products which are capable in providing them their health needs. But as the demand is increasing, proportionally their wastage is also increasing in the world. Processing

industries are causing lots of losses and wastage and unfortunately giving harm to nature. The waste and losses related to fruits and vegetables are very high compared with other foods this can reach a level of 60% if proper treatment is not given to them now. Out of the all wasted food crops and products, 25-30% comprises only wastage of fruits and vegetables which is surely a great amount. The waste related to fruits and vegetables contains their peels, seeds, skins, rind, and pomace, but in contrast, they contain lots of valuable compounds in them like dietary fibers, vitamins, antioxidants, oils, polyphenols, etc. Utilization of these waste peels, seeds, etc. will reduce their waste amount and will somehow give positive impacts on the environment. The nature of waste associated with banana is its peel which comprises 35% of wastage out of total production; Citrus fruits comprise 30-40% of wastage in the form of a rag, peel, seed; dragon fruit comprise 35-45% of waste in the form of rind, seeds; grapes comprise 20% waste in the form of skin, stem, seeds; papaya contains 20-30% waste as its peels, seeds; peas contain 35% of wastage in the form of a shell, etc. (Sagar et al., 2018)

"Banana" is an edible fruit belonging to the monocotyledonous family: Musaceae, Genus: *Musa*, scientifically known as; *Musa acuminata* is predominantly of Asian origin. banana peel is a rich source of phytochemical compounds than its pulp and so can be utilized in many fields like it is extremely useful in treating skin problems including rashes, irritations, and allergies, it can contribute to environmental problems by disposing of them in landfills, it can also use as a natural wrapping paper with the addition of essential oils, etc. It also gives relief from mosquito bites by rubbing banana skin in the affected area.Banana peel has antioxidants, antimicrobial, and also antibacterial properties against both gram-positive and gram-negative bacteria. Due to the presence of glycosides and alkaloids, it exhibits medicinal properties like helpful in treating bacterial infections like cough, fever, cold. Banana peels perform various pharmacological activities like healing wounds, extract from banana peels can prevent infections caused by *Salmonella typhi, E-coli, Staphylococcus aureus, Campylobacter, M.catarrhalis, E.aerogenes*. Along with this, it has anti-diabetic properties, anti-inflammatory, anti-psoriasis properties as well. (Prashanthi and Chaitanya, 2020)

"Papaya" belongs to the Caricaceae family, scientifically known as *Carica papaya* is a herbaceous plant and has a high demand as a natural bio-active compound containing not only fruit but also leaves, peels, seeds, roots, and flesh. Papaya peel generates a huge amount of waste so disposing of is not good for the environment hence, can be utilized in various fields to prevent wastage as can be useful in the cosmetic industry as it has anti-dandruff, moisturizing, skin smoothing ability. Papaya peel is also rich in polyphenols and phytochemical compounds including alkaloids and Ninhydrin in a good amount so comprises medicinal properties along with this it has antioxidant, antidiabetic, and antibacterial properties as well. (Rodrigo et al., 2018). Papaya peel actively showed antibacterial activity against gram-negative bacteria like *salmonella enteric typhi, Shigella flexneri, E.coli*, and also against gram-positive bacteria like *Staphylococcus epidermidis, Staphylococcus aureus, Listeria monocytogenes*. (Rohin et al.,2012)

MATERIALS AND METHODOLOGY

Collection of Sample

Fruit samples papaya and banana were taken from the local vendor; peeled off and let those peels dried in an incubator for 2 days at 37ºC. After that dried peel samples were converted into a fine powder with the help of a mechanical grinder.

Preparation of Extraction

Ethanol extraction of samples was done by taking a 3grm powdered sample in 10mL of 100% ethanol. Vigorously mixed and kept undisturbed for 24hrs. , then the supernatant was collected in 5mL vials. The collected extracts were stored in a refrigerator for future analysis.

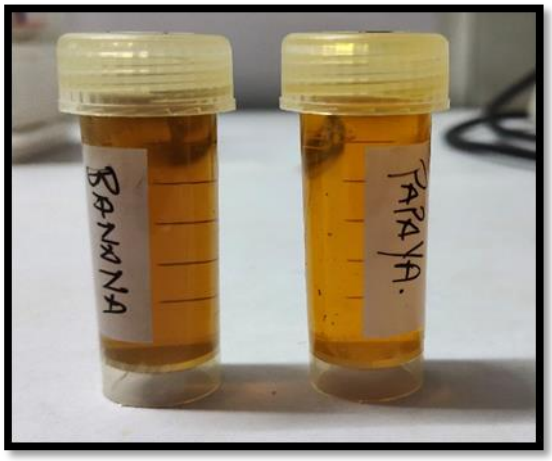


FIGURE 1; Extracted plant samples in 5ml vials

Qualitative Analysis of phytochemicals

Qualitative analysis of phytochemicals was done as per the protocol written below the tests (Kebedeet al., 2021; Roghini and Vijaylakshmi, 2018)

i. Saponin Test (Foam Test): - 2mL of plant extract was taken and diluted with 8mL of distilled water and shaken in a graduated cylinder for approx. 5-10min. The appearance of 1cm of foam layer indicates the presence of saponin in the samples.
ii. Tannin Test (Ferric Chloride Test): -1mL of plant extract was taken with 2mL of 5% Ferric Chloride ($FeCl_3$), the appearance of dark blue or greenish-black color indicates the presence of tannin in the samples.
iii. Steroid Test: - 1mL of plant extract was taken with 1mL of chloroform ($CHCl_3$) and 1mL of conc. Sulphuric acid (H_2SO_4). Brown ring formation indicates the presence of steroids in the samples.
iv. Phenol Test: -1mL of plant extract was taken with 500µL of ferric chloride ($FeCl_3$), the appearance of blue or green color indicates the presence of phenols in the samples.
v. Flavonoids Test (NaOH Test): -500µL of plant extract was taken with 500µL of 2N sodium hydroxide (NaOH), after the formation of yellow color 200µL of conc. Hydrochloric acid (HCl) was added; the disappearance of yellow color indicates the presence of flavonoids in the samples.
vi. Glycoside Test: -1mL of plant extract was taken with 1mL of chloroform ($CHCl_3$) and 1mL of 10% ammonia (NH_3) solution; the appearance of pink color indicates the presence of glycoside in the samples.
vii. Anthocyanin Test: -1mL of plant extract was taken with 1mL of 2N sodium hydroxide (NaOH), the mixture was heated for 5min. at 100°C.The appearance of bluish-green color indicates the presence of anthocyanin in the samples.
viii. Quinones test: -500µL of plant extract was taken with 250µL of conc. Sulphuric acid (H_2SO_4), the appearance of red color indicates the presence of quinone in the samples.
ix. Phlobatannin Test: -500µL of plant extract was diluted with 500µL distilled water and then 100µL conc. Hydrochloric acid (HCl) was added. The appearance of red precipitates indicates the presence of Phlobatannin in the samples.
x. Coumarin Test: -750µL of plant extract was taken with 1mL 10% sodium hydroxide (NaOH). The appearance of yellow color indicates the presence of coumarin in the samples.
xi. Anthraquinones Test: -500µL of plant extract was taken with 750µL of 10% ammonia (NH_3) solution. The appearance of pink color precipitates indicates the presence of anthraquinone in the samples.

xii. Alkaloid Test: -1mL of plant extract was taken with 75µL of conc. Hydrochloric acid (HCl) and 400µL of Dragendorff's reagent. The appearance of an orange-brown precipitate indicates the presence of alkaloids in the samples.

xiii. Anthocyanosides Test: -1mL of plant extract was taken with 2mL diluted hydrochloric acid (HCl). The appearance of pale pink color indicates the presence of anthocyanosides in the samples.

xiv. Ninhydrin Test: -500µL of plant extract was taken with 500µL ninhydrin reagent then heated for 5 min. the appearance of blue color indicates the presence of ninhydrin in the samples.

xv. Terpenoids Test (Salkowski Test): -500µL of plant extract was taken with 2mL chloroform ($CHCl_3$) and 1mL conc. Sulphuric acid (H_2SO_4). The appearance of red-brown color at the interface indicates the presence of terpenoids.

Isolation of bacteria

Isolation of bacteria was done from unpasteurized milk, Mueller Hinton Agar media was prepared for the best growth of bacteria. Media was autoclaved for 15min. at 121ºC cooled to 45-50ºC and poured into sterile Petri plate. The plates were allowed to solidify the media for the spreading of bacteria.Spreading of unpasteurized milk samples for bacterial growth was done with a help of spreading the loop uniformly in all directions. Incubate for 24 hours for bacterial growth. Transparent colonies of bacteria were formed after the incubationAfter the growth of bacteria, streaking was done with the help of loops, Incubate Petri plate for 24 hours for the growth.

Identification of bacteria

Identification of bacteria was done to identify the bacterial species and to know that the bacteria grown is food poisoning causing bacteria. General morphological and biochemical tests have been done by following the test procedure to identify the bacteria. Gram staining, motility test, casein hydrolysis test, nitrate reduction test, starch hydrolysis test, catalase test, citrate test, urease test, Methyl Red/ Voges-Proskauer test, Indole, Dextrose was done to suspecting the bacteria. (Shoaib et al., 2020; Aryal, 2018)

Disc Diffusion Method

Anti-bacterial screening is done by using the disc-diffusion method. to check the inhibition activity of plant samples. It is done to measure the ability of extract samples to inhibit bacterial growth. Antibacterial screening is an important step to justify that the sample extracts taken were successful in inhibiting the bacterial action. This test is done by preparing the Nutrient Agar Media and a stock solution of plant extract was taken. Bacteria were spread on the media plate with the help of a cotton swab. For negative control, the disc is taken with forceps and placed on the quadrant. For positive control, the disc is dipped in antibiotic cefoxitin and placed in the quadrant. For plant samples, discs were dipped in samples and placed similarly. Then, the Petri plate was kept in an incubator at 37°C for 24 hours. Inhibition zones were measured with the help of a ruler including a 6mm diameter of discs. (Mahomoodally et al., 2008; Atef et al., 2019)

RESULTS

Qualitative Phytochemical analysis result

Qualitative phytochemical results were observed and recorded in table 1 for both *Carica papaya* and *Musa acuminata* peel samples. Phytochemicals Saponins, tannins, flavonoids, quinones, alkaloids, ninhydrin, terpenoids were actively found compounds in both the peel samples extract.

Table 1; Result for qualitative Phytochemicals analysis

S.no.	QUALITATIVE TEST	*Carica papaya*	*Musa acuminata*
1.	Saponin	++	++
2.	Tannin	+	+
3.	Steroids	-	-
4.	Phenols	+	+
5.	Flavonoids	++	+++
6.	Glycosides	-	++
7.	Anthocyanin	+/-	+
8.	Quinones	+	+
9.	Phlobatannin	-	-
10.	Coumarins	+	+
11.	Anthraquinones	-	+/-
12.	Alkaloids	+++	++
13.	Anthocyanosides	-	+/-
14.	Ninhydrin	++++	+++
15.	Terpenoids	++	+++

(+ Positive, - Negative, +/- may be positive or negative)

Morphological and Biochemical tests result

Table 2 showing morphological and biochemical test results for bacterial identification to identify the food poisoning bacteria. Bacteria 1; gram-negative, motile, and has positive results for Nitrate reduction, catalase and citrate test, whereas for Bacteria 2; gram-positive, motile and positive for casein hydrolysis, nitrate reduction, starch hydrolysis, catalase, citrate, urease, VP, and dextrose.

Table 2; Morphological and Biochemical test result

S. No.	TEST	Bacteria 1	Bacteria 2
1.	Gram staining	Negative	Positive
2.	Motility Test	Motile	Motile
3.	Casein Hydrolysis Test	Negative	Positive
4.	Nitrate Reduction Test	Positive	Positive
5.	Starch Hydrolysis Test	Negative	Positive
6.	Catalase Test	Positive	Positive
7.	Citrate Test	Positive	Positive
8.	Urease Test	Negative	Positive
9.	Methyl Red (MR)	Negative	Negative
10.	Voges-Proskauer Test (VP)	Negative	Positive
11.	Indole Test	Negative	Negative
12.	Dextrose Test	Negative	Positive

Disc diffusion method results

Figure 2 is observed as the creation of zones by the samples against bacteria. Regarding these zones, the inhibition zone created by the peel sample extract was measured and recorded in Tables 3 and 4 for *Campylobacter jejuni* and *Bacillus cereus* respectively. With the help of recorded tables graph, 1 and 2 was plotted for the same to clearly distinguish the areas being formed.

For *Campylobacter jejuni*For *Bacillus cereus*

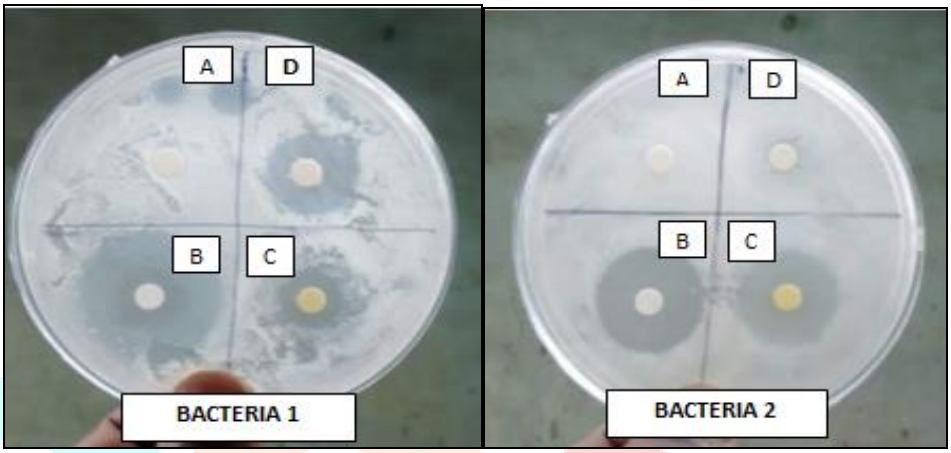


FIGURE 2; Antibacterial activity of plant samples against bacteria

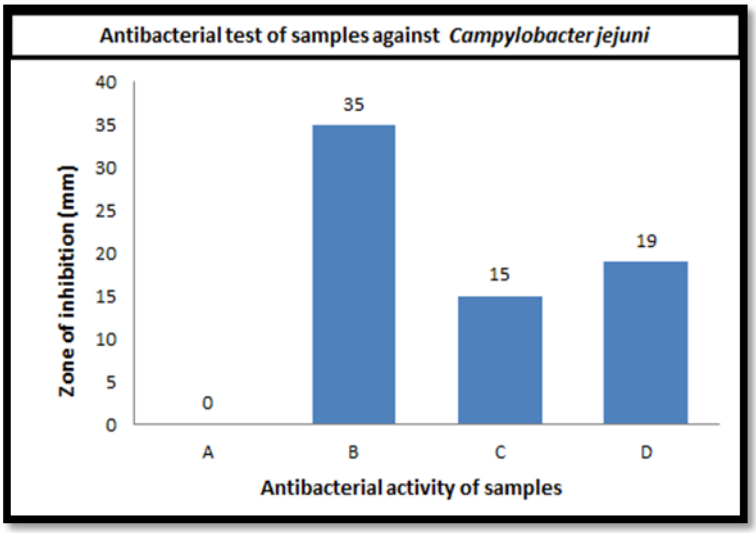
Table 3; Zone of inhibition of samples against bacteria 1*(including 6mm diameter of discs)

Quadrant	Zone of inhibition (mm)*
A; Negative control	0
B; Positive control	35
C; *Carica papaya*	15
D; *Musa acuminata*	19

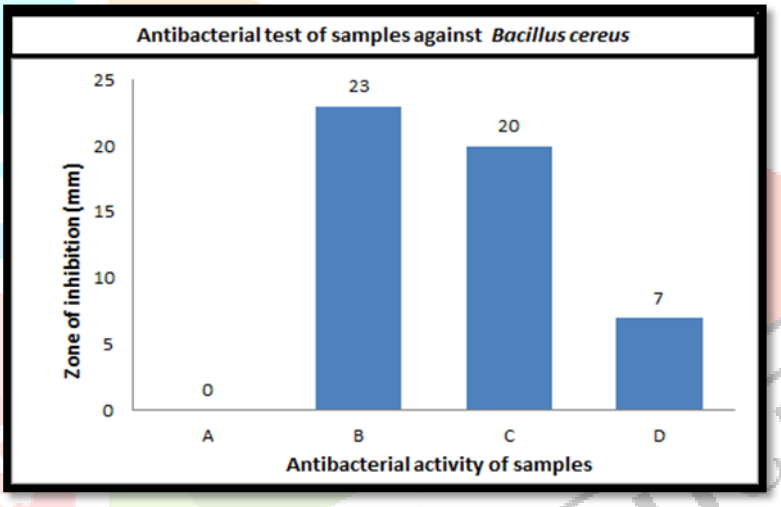
Table 4; Zone of inhibition of samples against bacteria 2*(including 6mm diameter of discs)

Quadrant	Zone of inhibition (mm)*
A; Negative control	0
B; Positive control	23
C; *Carica papaya*	20
D; *Musa acuminata*	7

Graph 1; Graphical representation of zone of inhibition



Graph 2; Graphical representation of zone of inhibition



DISCUSSION

Following the above-recorded results for phytochemical analysis, identification of bacteria, and antibacterial screening, it can be defined that the presence of alkaloids, tannins, saponins, flavonoids are responsible for antibacterial activity in any plant extract sample. (Mahomoodally et al., 2008; Silva et al.,2016). The peel samples have excellently shown the presence of antibacterial phytochemicals in them which justifies that they can inhibit bacterial activity. Isolation and identification of bacterial cultures were done as per the protocols and through which bacteria 1 was identified as *Campylobacter jejuni* and Bacteria 2 was identified as *Bacillus cereus* after observing the results of the morphological and biochemical test. (Aryal,2018; Solanki et al.,2019). The antibacterial screening was observed as 15mm and 19mm for *Carica papaya* and *Musa acuminata* respectively against *Campylobacter jejuni* and 20mm and 7 mm against *B.cereus.* The peel samples have shown better action against *Campylobacter jejuni* than *Bacillus cereus.* The sample extract *Carica papaya* alone has good inhibition action against *Bacillus cereus,* The antibacterial action of samples developed because of the presence of antibacterial phytochemicals in them, presence of these compounds inhibit the pathogenic activity and also gives health benefits to the body.

CONCLUSION

Among the most popular fruit peel *Carica papaya and Musa acuminata* majorly contain antioxidants as well as antibacterial property in them. Both the taken fruit peel samples successfully have inhibition properties against identified bacteria so individually or synergistically will demonstrate positive results. Fruit and vegetable peel's wastage leads to many environmental problems hence, utilizing them for something good will have a great impact in the future. Food poisoning is a very common and serious issue in every country, caused by eating any unhygienic food following this pathogenic bacterium enters the body through food, grows and multiply and leads to the foodborne disease. So, slowing down the pathogenic activity is important to overcome the disease. Formulating any product by incorporating these fruit peels may help get rid off of the disease due to their antibacterial action and phytochemicals presence action. A phytochemical test was done and as per the tests they both have healthy phytochemicals compounds present in them and good quantity as well. These phytochemicals play a vital role in the human body and have many benefits like resistance to bacterial, fungal infections, boost the immune system, give positive effects on the body, apart from this phytochemicals are also control diabetes, high blood pressure, etc. Introducing any nutraceutical or medicinal product containing *Carica papaya and Musa acuminata* peels will help treat food poisoning illness or any other illness caused by these bacteria. The demand for effective medications for any disease is always highest, so establishing any source of treatment containing an ingredient that not only giving heath relating benefits and relief from illness but also favoring environmentally friendly conditions is undoubtedly a good initiative for the future.

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