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In search of natural preservatives: Evaluation of *Artocarpus heterophyllus* lam leaves extracts against food spoilage microorganisms

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Abstract
As a result of the rapid increase in human population, global food insecurity has emerged as a significant issue. Due to numerous health risks, it is necessary to develop safe natural preservatives in order to stop the spread of food poisoning mechanisms. It ought to be safe for people and animals as well as the environment. The goal of the current study was to evaluate the potential antimicrobial activity of *Artocarpus heterophyllus* Lam leaf extracts in petroleum ether, chloroform, acetone, ethanol and water against various food spoilage bacteria (*Escherichia coli*, *Bacillus cereus*) and fungi (*Aspergillus niger* HN-2).The antimicrobial activities and minimum inhibitory concentrations (MIC) of various plant extracts were determined using the agar well diffusion method. The experimental results clearly demonstrated its ability to limit the growth of tested microorganisms that are commonly responsible for food poisoning and spoilage. A petroleum ether extract showed inhibition of *Bacillus cereus* with a MIC value of 60 mg/ml, but did not inhibit *Escherichia coli* or *Aspergillus niger* HN-2 growth. Similarly, acetone extract showed inhibition of *Escherichia coli* with an MIC value of 80 mg/ml but was ineffective against *Bacillus cereus* and *Aspergillus niger* HN-2. In conclusion, the researchers will be engaged in the future to discover cutting-edge strategies for preventing food microbes to stop food spoilage through natural ways.

Keywords: Food spoilage, jackfruit, extracts, MIC, phytochemical screening, antimicrobial

Introduction
Food spoilage refers to undesirable and unacceptable changes in food products that render them unfit for human and animal consumption. Food spoilage caused by microorganisms affects the entire world, including developed countries, resulting in food wastage and food poisoning, posing a dreadful public and economic health issues for the society [1-3]. The use of chemical substances commonly referred to as 'preservatives' is one of many methods for inhibiting the growth of undesirable microorganisms in order to prevent food spoilage and ultimately, poisoning[4]. Food spoilage is frequently caused by a variety of microorganisms, including fungi like *Aspergillus niger*, gram positive bacteria like *Staphylococcus aureus*, *Bacillus cereus* and gram negative bacteria like *Salmonella typhi*, *Escherichia coli*, and *Pseudomonas aeruginosa*.
Mostly the *Escherichia coli*, rod-shaped gram negative bacteria, are harmless and act as good bacteria for our digestive system but some strains have been reported the outbreaks linked to eating undercooked meats representing the most dangerous pathogens in food products because of its capacity to survive in an acidic environment of the GI [5, 6]. *Aspergillus niger*, a type of mold, has been linked to the cause of 'black mold' on certain foods, such as apricots, onions, grapes, and so on, making it a food spoilage organism [7]. *Bacillus cereus* is generally considered to produce acute intoxication resulting diarrhoeal syndrome and vomiting due to its toxic compounds. It relates to the frequent cause of gastroenteritis [8-10].
Even though chemical preservatives have demonstrated their effectiveness in preventing the growth of such undesirable microorganisms, newer approaches are still needed to prevent their unpleasant side effects and the rising threat of microbial resistance. Due to their relative safety, researchers are concentrating on using plant extracts with antimicrobial activities as preservatives for these purposes [11].

Artocarpus heterophyllus Lam, also known as 'jack-tree' or 'jackfruit tree' belonging to family Moraceae is evergreen tree, grows to a height of 10-25 m, native to India and widely distributed to the large area of tropical and sub-tropical countries like Indonesia, China, Brazil, Sri Lanka and Thailand [12]. The fruit is also famous as food of poor's due to its easy availability and inexpensiveness [13]. Traditionally, every part of the drug is found useful for the curing of ailments like leaves for boils and wounds, young fruits as carminatives, ripe fruits as brain tonics and aphrodisiac, wood as hypoglycemic, seeds as diuretic and roots as Antiasthmatic [14, 15].

Since jackfruit leaves are rich in plenty of nutrient substances including alkaloids, tannins, flavonoids, phenolic compounds, proteins, carbohydrates, fats, calcium, manganese and iron [16, 17]; our main objective for this study was to investigate *A. heterophyllus* leaf extracts against food spoilage microorganisms namely *Bacillus cereus*, *Escherichia coli* and *Aspergillus niger* and to evaluate the group of phytochemicals responsible for it.

Materials and Methods

Materials

Petroleum ether, chloroform and acetone were procured from CDH Fine Chemical, India. Nutrient agar from HiMedia, India and ethanol of AR grade from Changshu Hongsheng Fine Chemical, China, were used. For the antimicrobial activity, the sterile water was used while for the extraction purposes own laboratory made double-distilled water was used.

The microbial strains were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh, India to assess the antimicrobial activity of leaves extracts of *A. heterophyllus* against food-borne pathogenic and spoilage microorganisms. Gram positive bacteria, *Escherichia coli* (MTCC 42), Gram negative bacteria, *Bacillus cereus* (MTCC 12856) and Fungus, *Aspergillus niger* HN-2 (MTCC 11098) were used in this study (Figure 1).

Plant leaves were collected from the Apollo College botanical garden in Anjora, Chhattisgarh (21.19664˚ N, 81.21988˚ E). The Department of Botany, Govt. T.V.Y.PG. Autonomous College, Durg (C.G.), Chhattisgarh, confirmed the taxonomical authentication of said plant material. After collecting the plant material, it was carefully cleaned with water to get rid of any remaining dust and undesirable materials. It was then allowed to dry in the shade and ground into a fine powder using a 100 mm sieve.

Fig 1: Microbial cultures used- A: *Bacillus cereus*; B: *Escherichia coli*; C: *Aspergillus niger* HN-2

Plant extraction and phytochemical testing

The powdered plant material was extracted over the course of seven days using petroleum ether, chloroform, acetone, ethanol, and water using a Soxhlet apparatus. To recover the organic solvents, the filtered liquid extracts were subsequently evaporated in a rotary vacuum evaporator. After the extracts were further concentrated, they were dried in a water bath and their contents were examined for the presence of amino acids, proteins, carbohydrates, steroids, alkaloids, saponins, anthraquinone glycosides, flavonoids, and terpenoids as well as for their antimicrobial properties. Various standard procedures were employed for the phytochemical screening [18, 19].

Chemical tests for carbohydrates

1. **Molisch's test:** Add a few drops of alpha-napthol solution in alcohol to 2-3 ml of aqueous extract, shake,

and add conc. H_2SO_4 from the sides of the test tube. At the intersection of two liquids, a violet ring forms.

2. **Fehling's test:** Boil 1 ml Fehling's A and 1 ml Fehling's B solution for 1 minute. Add an equal amount of test solution. Following 5-10 minutes in a boiling water bath, precipitation appears to be yellow at first, then brick red.
3. **Barfoed's test:** In a test tube, combine an equal volume of Barfoed's reagent and test solution. On 5 minutes in a boiling water bath, depending on the amount of reducing sugar in the test solution, the solution appears green, yellow or red.
4. **Bial's Orcinol test:** Add a few drops of the test solution to the boiling Bial's reagent. It has a green or purple tint on presence of pentose sugars.
5. **Selwinoff's test:** On a water bath, heat 3 ml Selwinoff's regent and 1 ml test solution for 1-2 minutes. Red color develops on presence of hexose sugars.
6. **Tollen's phloroglucinol test:** Combine 2.5 ml of concentrated HCl and 4 ml of 0.5% phloroglucinol. Add 1-2 ml of the test solution. Warm up the mixture. The color changes from yellow to red on presence of hexose sugars.

Chemical tests for proteins

a) Biuret test

Add 4% NaOH and a few drops of 1% $CuSO_4$ solution to 3 ml of test solution. The color violet or pink appears.

b) Million's test

Combine 5 ml of Million's reagent with 3 ml of test solution resulting white precipitation. On warming the precipitate turns brick red or dissolves to produce a reddish-colored solution.

c) Test for sulphur containing proteins

Combine 2 ml of 40% NaOH solution, 2-3 drops of 10% lead acetate solution and 5 ml of test solution. Boil. Lead sulfide forms in the solution, which causes it to turn black or brownish.

Chemical tests for alkaloids

For alkaloidal testing, 1 g of dry extracts was combined with 2 ml of diluted HCl, shaken well, and then used for the subsequent tests.

a) Mayer's test

Add 1 ml of Mayer's reagent to 3 ml of the filtrates. The presence of alkaloids is indicated by the creamy precipitate.

b) Wagner's test

Add 1 ml of Wagner's reagent to 3 ml of the filtrates. Alkaloids are present as evidenced by the reddish brown precipitate.

c) Hager's test

Add 1 ml of Hager's reagent to 3 ml of the filtrates. The presence of alkaloids is indicated by the yellow precipitate.

d) Dragendroff's test

Add 1 ml of Dragendroff's reagent to 3 ml of filtrates. The presence of alkaloids is indicated by the presence of orange brown precipitate.

Chemical tests for saponins

a) Foam test

Shake a small amount of extract with water. The presence of saponins is confirmed by the persistent foam after 10 minutes.

b) Haemolysis test

On the slide, combine a small amount of extract with the blood. The saponins glycosides are represented by the hemolytic zone.

Chemical test for anthraquinone glycosides

Borntrager's test

Add diluted H_2SO_4 solution to 3 ml of the extract, boil and filter. Add an equal volume of benzene or chloroform to the cold filtrate. Shake firmly. Make the organic solvent separate. Add ammonia. Layer of ammonia turns pink or red.

Chemical tests for steroids

a) Salkowski's test

Add 2 ml of chloroform and 2 ml of concentrated H_2SO_4 to the 2 ml test solution. Shake firmly. The chloroform layer is red and the acid layer fluoresces a greenish yellow color.

b) Legal's test

Add 1 ml of sodium nitroprusside solution, 1 ml of pyridine, and 1 ml of test solution. A pink to red color is visible.

Chemical tests for terpenoids

a) Salkowski's test: See 'chemical test for steroids'.

b) Liebermann-Burchard's test: Add few ml of acetic anhydride to the extract dissolved in chloroform. Heat it and cool. Add few drops of concentrated H_2SO_4 from the side of the test-tube. Blue colour forms on presence of terpenoids.

Chemical tests for flavonoids

a) Shinoda test

Add a few drops of concentrated HCl and 0.5g of magnesium turnings to 5 ml of 95% v/v ethanol. Flavonoids are represented by the pink, crimson or magenta colour.

Chemical tests for tannins

a) Ferric chloride test

Dark green or deep blue color is produced by extract when mixed with 5% ferric chloride solution.

b) Lead acetate test

To extract, use a 10% w/v solution of basic lead acetate in distilled water. Precipitate has been obtained.

c) Potassium dichromate test

Potassium dichromate solution produces a dark precipitate when combined with the extract.

d) Gelatin test

Add a 1% w/v gelatin solution in 10% sodium chloride water. The presence of tannins is indicated by the presence of white precipitate.

Chemical tests for amino acids

a) Ninhydrin test: In a boiling water bath, heat 3 ml test solution and 3 drops 5% ninhydrin solution for 10 minutes. The color purple or bluish appears

Antimicrobial assay and minimum inhibitory concentration (MIC)

For the antimicrobial assay, the agar well diffusion method was employed. Sterilized nutrient agar medium plates were disseminated with a bacterial and fungal suspension (10^6 CFU/ml), prepared in sterile water, using a sterile glass spreader. Additionally, the holes were pierced with a sterile cork borer, and each bore was filled with 50 µl of extract that had been made in sterile water. Sterilized water was used here as the control. For *Bacillus cereus* and *Escherichia coli*, the plates were incubated for twenty-four hours at 35-37 °C; for *Aspergillus niger* HN-2, the incubation period was 96 hours.

The minimum inhibitory concentration (MIC) is the lowest amount of an antimicrobial that can prevent visible microbial growth after being incubated for one night. The initial extract concentration of 50 mg/ml was evaluated for growth inhibition in order to determine the MIC's of extracts. Additionally, lower concentrations for those extracts that displayed an inhibition zone were examined, whereas concentrations were raised for extracts that did not. The concentrations used for the MIC's of extracts were typically between 10-100 mg/ml. The test was conducted thrice, and the mean and standard deviation of the inhibition zone diameter, expressed in millimeters, for every extract were noted.

Results and Discussion

Artocarpus heterophyllus Lam, a member of the family Moraceae, was identified as the plant material employed in the current study to evaluate its efficacy as an antibacterial agent against food-borne pathogenic and spoilage microorganisms. For future use, a herbarium specimen (ACP/HER/M.Ph/01/22) was placed at the Govt. TVYPG College, Durg, Chhattisgarh and the Pharmacognosy Department of the Apollo College of Pharmacy, Durg, Chhattisgarh.

Following the phytochemical analysis of extracts, it was discovered that proteins and carbohydrates were present in the ethanol and aqueous extracts, while alkaloids, saponin glycosides, and terpenoids were present in all extracts with the exception of ethanol. Flavonoids in the petroleum ether and aqueous extract, tannins in all extracts and steroidal components in the chloroform and petroleum ether extracts were reported. All studied extracts lacked anthraquinone glycosides and amino acids (Table 1).

Table 1: Phytochemical screening

Phytoconstituents	Tests	Petroleum ether extract	Chloroform extract	Acetone extract	Ethanol extract	Aqueous extract
Carbohydrates	Molisch's	-	-	-	+	+
	Fehling's	-	-	-	+	+
	Barfoed's	-	-	-	+	+
	Bial's Orcinol	-	-	-	+	+
	Selwinoff's	-	-	-	+	+
	Tollen's phloroglucinol	-	-	-	+	+
Proteins	Biuret	-	-	-	+	+
	Million's	-	-	-	+	+
	Sulphur containing	-	-	-	+	+
Saponin glycosides	Foam	+	+	+	-	+
	Haemolysis	+	+	+	-	+
Alkaloids	Mayer's	+	+	+	-	-
	Wagner's	+	+	+	-	-
	Hager's	+	+	+	-	-
	Dragendroff's	+	+	+	-	-
Anthraquinone glycosides	Borntrager's	-	-	-	-	-
Steroids	Salkowski's	+	+	-	-	-
	Legal's	+	+	-	-	-
Flavonoids	Shinoda	+	-	-	-	+
Terpenoids	Salkowski's	+	+	+	-	+
	Liebermann-Burchard's	+	+	+	-	+
Tannins	Ferric chloride	+	+	+	+	+
	Lead acetate	+	+	+	+	+
	Potassium dichromate	+	+	+	+	+
	Gelatin	+	+	+	+	+
Amino acids	Ninhydrin	-	-	-	-	-

The antimicrobial assay demonstrated that some extracts were effective against the food-borne pathogenic and spoilage bacteria and fungus examined (Figure 2). The antimicrobial assay certainly shown that the petroleum ether and acetone extracts limit the growth of at least one microbial strain. Furthermore, a petroleum ether extract of *Artocarpus heterophyllus* inhibited *Bacillus cereus* (inhibition zone - 30 mm) with a MIC value of 60 mg/ml, but did not inhibit *Escherichia coli* or *Aspergillus niger* HN-2 growth up to 100 mg/ml. Similarly, acetone extract inhibited *Escherichia coli* (inhibition zone - 19 mm) with an MIC value of 80 mg/ml but was ineffective against *Bacillus cereus* and *Aspergillus niger* HN-2 up to 100 mg/ml. All three of the examined microorganisms showed no special inhibition by chloroform, ethanol or aqueous extracts up to a concentration of 100 mg/ml, it was revealed (Table 2).

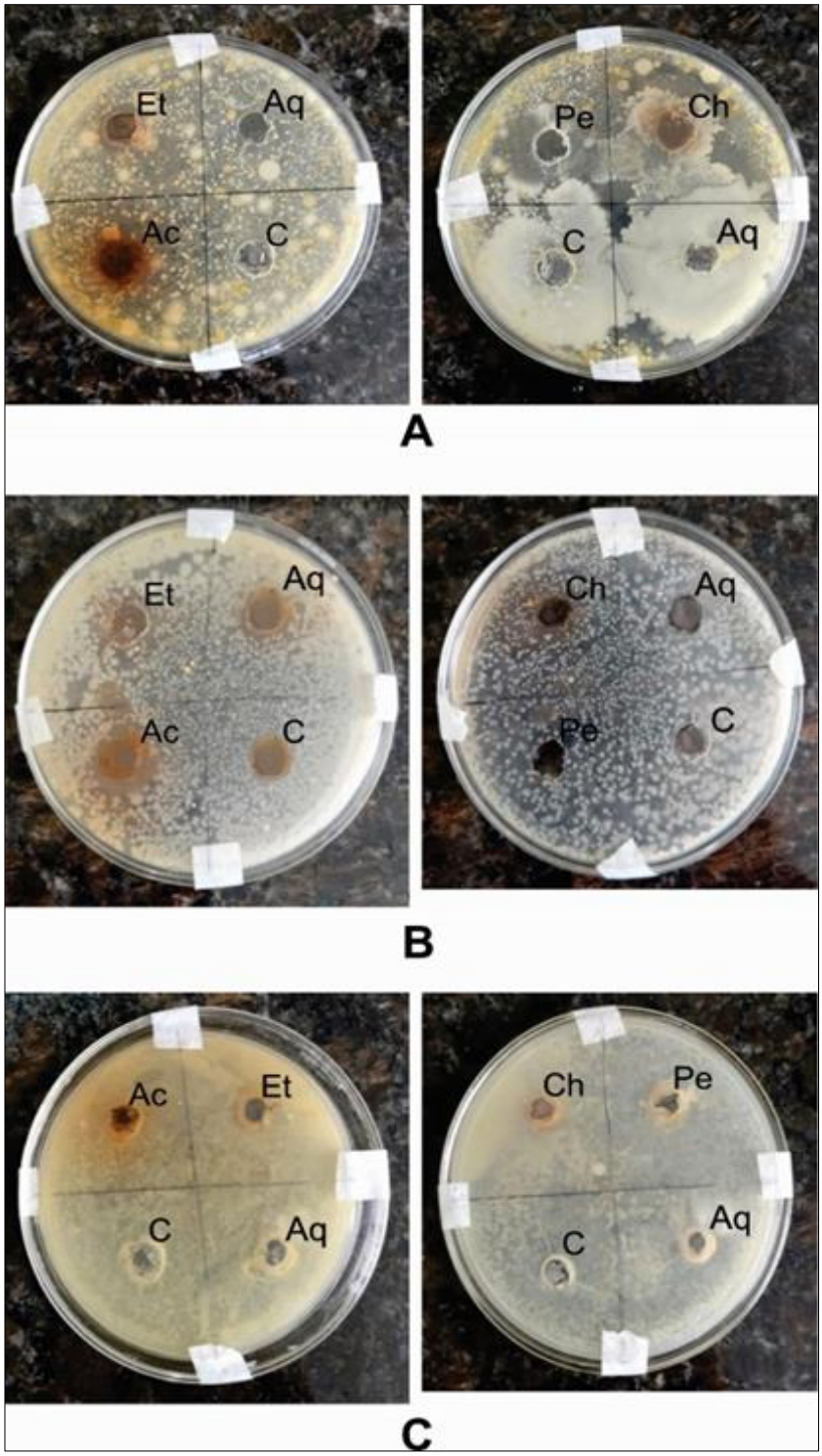

Fig 2: Antimicrobial assay against(A)- *Bacillus cereus*, (B)- *Escherichia coli* and (C)- *Aspergillus niger* HN-2; Et- Ethanolic extract, Aq- Aqueous extract, Ac- Acetone extract, Ch- Chloroform extract, Pe- Petroleum ether extract and C- Control

Table 2: Antimicrobial assay and MIC

Extracts	Minimum Inhibitory Concentration (MIC)		
	Bacillus cereus	***Escherichia coli***	***Aspergillus niger* HN-2**
Petroleum ether	60 mg/ml	> 100 mg/ml	> 100 mg/ml
Chloroform	> 100 mg/ml	> 100 mg/ml	> 100 mg/ml
Acetone	> 100 mg/ml	80 mg/ml	> 100 mg/ml
Ethanol	> 100 mg/ml	> 100 mg/ml	> 100 mg/ml
Aqueous	> 100 mg/ml	> 100 mg/ml	> 100 mg/ml

So, the current study found that acetone extract and petroleum ether extract of *Artocarpus heterophyllus* Lam. are effective at inhibiting food-borne pathogenic and spoilage bacteria.

Conclusion

Nowadays, widespread issues like food contamination and spoilage pose a threat to people's health. Utilizing artificial preservatives allowed us to control this predicament thanks

to modernity and scientific thinking. However, due to increased microorganism resistance to such preservatives and potential toxicity, we need a superior solution that may be effective against them. Researchers are constantly researching the use of plant extracts with antibacterial action as preservatives in an effort to find better alternatives. The antibacterial activity of *A. heterophyllus* leaf extracts was assessed in this work against the bacteria *Bacillus cereus*, *Escherichia coli*, and *Aspergillus niger* HN-2, which are mostly responsible for food poisoning and spoiling. Acetone and petroleum ether extracts were found to be effective against *Bacillus cereus* and *Escherichia coli* respectively. Although the effective concentration of the extracts was found to be higher, it is already known that the extract is a mixture of many different constituents. If we could further process, in particular, the fractional separation assisted isolation of specific constituents of said petroleum ether and acetone extract, the breakthrough molecule as a natural preservative could be discovered.

Conflict of interest

The authors declare that there is no conflict of interest.

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