A study on antibacterial efficacy of different extracts of *Artocarpus chama* fruits and identification of bioactive compounds in the most potent extract

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ABSTRACT

This study is designed to evaluate the antibacterial potential of various extracts of mature green fruits of *Artocarpus chama* with the identification of bioactive compounds. The antibacterial efficacy was tested against eight pathogenic bacteria using standard antibiotics as the positive control. Antibacterial bioassay was performed by measuring zone of inhibition, Minimum Inhibitory Concentrations (MIC), and Minimum Bactericidal Concentrations (MBC). The most effective extract was further subjected to preliminary phytochemical tests, Thin Layer Chromatography (TLC), and Fourier Transform Infrared analysis. Ethyl acetate extract (EAAC) was found to be the most effective extract producing an inhibition zone of 45.50 ± 0.50 mm against *Pseudomonas aeruginosa* MTCC 2453. Effective spots (ES) (Rf=0.37) of EAAC separated by TLC analysis were subjected to indirect contact bio-autography for evaluating their efficacies against the tested bacterial strains. Values of MBC/MIC for the extract ranged from 1.20 to 1.80 (<2) indicating their bactericidal property. Gas Chromatography-Mass Spectroscopy analysis of ES was performed for the identification of bioactive antibacterial compounds. Alkaloids, saponins, steroids, terpenoids, and an array of functional groups in EAAC were detected. MICs of ES ranged from 6 - 10 µg/ml. Four bioactive compounds in ES were detected in GC-MS analysis. Thus, this study revealed that ethyl acetate extract of *A. chama* fruits contained several bioactive compounds with promising bactericidal properties against tested strains of both Gram-positive and Gram-negative pathogenic bacteria.

Keywords: *Artocarpus chama;* Bactericide; Minimum Inhibitory Concentration; FT-IR analysis; GC-MS analysis; Bioactive compounds.

1. INTRODUCTION

Treatment of bacterial infections has been proven to be a crucial problem in recent times. Immuno-compromised patients and medical professionals are facing increasing risks of nosocomial infections with significant morbidity and mortality. The rapidly rising incidence of these nosocomial infections is a major problem worldwide[1]. At present, the principal way to combat bacterial infection is the application of antibiotics. However, the emergence of multidrug-resistant strains of microorganisms renders a threat to the currently available drugs for the treatment of a wide variety of microbial infections and diseases[2-3]. Hence, researchers are showing an increased interest in finding plant-derived drugs or phytochemicals for treating such diseases. World Health Organization is keenly interested in the use of herbal medicines in developing countries[4-5]. As antimicrobial agents, plant products are safe and effective alternatives to commercially available antibiotics[6-8]. A special characteristic of higher-order plants is having the capacity to produce a large number of secondary

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metabolites [9-10]. Compounds obtained from plants for their pharmacological assay have been a rich source of innumerable therapeutic agents and about 44% of chemicals present in semi-synthetic drugs are of plant origin[11].

Artocarpus chama Buch. (Synonym *A. chaplasha* Linn)[12] is a tall deciduous tree of the Moraceae Family and grows all over India and South Asian countries. Organic and aqueous soluble fractions of leaves were assessed for antioxidant, thrombolytic, cytotoxic, membrane stabilizing, and antimicrobial activities[13-14]. Anti-oxidant and anti-alpha amylase activities of *A. chama* fruits were reported earlier by Paul and Sikdar (2011)[15], whereas *in vitro* antioxidant potential of *fruits of A. chama* Buch. was evaluated by Ahmed et al. (2012)[16]. A review of the literature reveals that there is no published data to ascertain the fruits of this plant as a source of antimicrobial agents.

Therefore, it is worthwhile to screen *in vitro* antibacterial potential of fruit and also to identify and characterize its phytoconstituents. The present study focuses on the evaluation of *in vitro* antibacterial properties of several extracts of green fruits of *A. chama*, phytochemical characterization of the most effective extract, isolation and identification of active ingredients and to determine whether it is bactericidal or bacteriostatic.

2. Materials and Methods:

2.1. Collection of plant material:

Fresh mature green fruits of *A. chama* were collected randomly during May-June, 2019 from the outskirts of Burdwan town, Burdwan (23°16´N, 87°54´E), West Bengal, India. The plant was taxonomically authenticated by a plant taxonomist, Professor Ambarish Mukherjee, Department of Botany, The University of Burdwan, Burdwan. A voucher specimen of the plant was deposited (Voucher specimen no. GCZSB-014) in the herbarium of our laboratory for future reference.

2.2. Test microorganism:

Eight pathogenic bacterial strains were taken for the present study from Mosquito, Microbiology, and Nanotechnology Research Units, Parasitology Laboratory, Department of Zoology, The University of Burdwan, of which *Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 2940, *Escherichia coli* MTCC 739 and *Pseudomonas aeruginosa* MTCC 2453 were human pathogens and *P. putida* MTCC 1654, *P. fluorescens* MTCC 103, *B. mycoides* MTCC 7343 and *B. licheniformis* MTCC 530 were fish pathogens. All the bacteria were periodically sub-cultured in Nutrient Broth (Hi-Media M002) at 37˚C and those were maintained at a temperature of 4˚C on Nutrient Agar (Hi-Media M012) slants.

2.3. Chemicals:

Chemicals required were Nutrient Broth, Nutrient Agar, Mueller Hinton Broth, Mueller Hinton Agar, Dimethylsulphoxide, Potassium bromide (IR spectroscopy grade), 2, 3, 5- triphenyltetra-zolium chloride, and $FeCl_3$, which were brought from Hi-Media Laboratories Pvt. Limited, Mumbai, India. Petroleum ether, ethyl acetate, and acetone were purchased from MERCK Specialities Pvt. Ltd., Mumbai, India. Silica Gel G for preparatory TLC plates was bought from Sisco Research Laboratories Pvt. Ltd. Taloja, Maharashtra, India. All the chemicals including the solvents used in this study were of analytical reagent grade and the highest quality available in the market.

2.4. Preparation of crude extract:

The fruits were first rinsed thoroughly in tap water and then in distilled water to remove dirt and dried in a paper towel. Cleaned fruits were cut into small pieces and blended by an electrical blender and the liquid obtained was subjected to filtration using Whatman's no-1 filter paper. The filtrate of the blended fruits was considered as the stock crude solution (100% concentration) and stored in a refrigerator at 4˚C for further experiments.

2.5. Preparation of solvent extract:

For solvent extraction, green fruits were cut into small pieces and air-dried at room temperature for 14 days. Dry pieces of fruits were then transferred into the thimble of the Soxhlet apparatus for successive extraction with three solvents namely petroleum ether, ethyl acetate, and acetone one by one for 72 h each with maximum 8 h

extraction per day. The volume of each solvent to the weight of the dried fruits was kept in a fixed ratio of 10:1. Extracts were evaporated to dryness and stored at 4˚C until tested and analyzed.

2.6. Preparation of inocula:

Freshly prepared inocula were used. About18 hour broth cultures of the test bacteria were suspended into sterile Müeller-Hinton Broth (pH 7.4.) (MHB). Those were standardized with the gradual addition of normal saline to compare their turbidity to McFarland standard of 0.5 which is approximately 1.0×10^8cfu/ml with the help of a spectrophotometer.

2.7. Agar well diffusion method:

Crude and solvent extracts of *A. chama* fruits were examined for their antibacterial activities by *in vitro* agar well diffusion method[17-18] based on the area of inhibition zones. Autoclaved Nutrient agar was poured on Petri plates and allowed to solidify. Plates were then inoculated with 100 µl of the inocula of tested bacteria by a sterile spreader. Equidistant wells were made on the plates with a sterile cork borer of 6 mm diameter. Approximately 50 µl of crude (100%) and solvent extracts (10 mg/ml) were introduced into the wells. As 1% (v/v) dimethylsulphoxide (DMSO) was used to dissolve the powdered solvent extracts, it was taken as a negative control for solvent extracts. Sterile distilled water was used as a negative control for crude extract. The plates were then incubated at 37°C for 24 h. The positive results (sensitivity) were determined at the end of the incubation period by the presence of a clear zone of inhibition around active extracts and measured in millimeters (mm). Two diameter readings, perpendicular to each other, were taken for each zone[19]. The average readings were considered as final measurements of the diameters of the inhibition zones. The above method was carried out thrice and the mean of the results was taken.

2.8. Antibiotic susceptibility test:

The test on each bacterial strain was done with commercially available antibiotics (Hi-Media Laboratories Pvt. Limited, Mumbai, India) of varying concentrations by using the agar disc diffusion method as recommended by the National Committee for Clinical Laboratory Standard[20] (NCCLS, 2000). The standard culture of each strain was spread on the agar surface by a sterile glass spreader. After inoculation of the bacterial strains, antibiotic discs were placed over the agar plates using sterile forceps maintaining equal distance from each other and kept at 37°C for 24 h.

2.9. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Ethyl acetate extract (EAAC):

MIC of ethyl acetate extract (EAAC) was determined by the broth dilution method. One mg per ml of extract (stock solution) was subjected to dilutions so that the concentrations would range between 400 µg/ml and 3.125 µg/ml (i.e., 400, 350, 300, 250, 200, 175, 150, 125, 100, 75, 50, 25, 12.5, 6.250 and 3.125 µg/ml) and each concentration of the extract was pipetted into test tubes with Müeller-Hinton Broth (MHB), where each tube contained total 500 µl of solution. Each of these concentrations of the extract was mixed with 10 µl 1.0×10^6cfu/ml bacterial suspension in test tubes. The tubes were incubated at 37°C for 24 h. MIC was taken as the lowest concentration of the extract that inhibited the growth of the bacteria. MBC was determined by sub-culturing 10 µl of the MIC tube solution (showing no visible growth) on a fresh Müeller-Hinton Agar (MHA) plate and incubated for 24 h at 37°C. The concentrations of the extract used for this purpose ranged between 150 µg/ml and 500 µg/ml (i.e., 500, 450, 400, 350, 300, 250, 200, 175 and 150 µg/ml). The highest dilution that yielded no bacterial growth on the inoculated plates was taken as MBC.

2.10. Preliminary phytochemical analysis of EAAC:

EAAC was subjected to preliminary phytochemical screening following standard protocols for the presence of tannins, flavonoids, saponins, glycosides, terpenoids, alkaloids, and steroids[21-23].

2.11. Fourier Transform Infrared analysis of EAAC:

Fourier Transform Infrared Spectrophotometer is a potential tool for the identification of functional groups in

plant extracts. Chemical bonds of the molecule can be identified through the interpretation of the infrared absorption spectrum. To prepare translucent pellets for FTIR analysis, 10 mg of the dried extract powder (EAAC) was encapsulated in 100 mg of Potassium bromide (KBr) by using the Hydraulic Press apparatus. KBr pellet without extract was considered as control. Prepared pellets were scanned in an FTIR spectrometer (Jasco, FT/IR- 4700). For this instrumental analysis scan range was from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.12. Indirect Contact Bio-autography test:

EAAC was resolved by thin-layer chromatography using silica gel plates (TLC Silica gel 60 F_{254} plates, 20×20 cm) with developing mobile phases containing different ratios of petroleum ether and ethanol (2:1) producing visible spots on TLC plates. The inoculum of each tested bacterium (1×10^6 CFU/ml) was sprayed onto those TLC plates and was subjected to incubation in a dark chamber at a temperature of 25˚C for 48 h under humid conditions. After incubation, the TLC plates were sprayed with aqueous 2, 3, 5- triphenyltetra-zolium chloride (TTC) for proper visibility of the zones[24] and further incubated for 24 h at 25˚C. Clear zones i.e. emergence of creamy white color areas around the purple-red background on the agar surface corresponding to the spots in TLC plates indicated the presence of active compound/s with antibacterial activity. Rf value of the effective spots (ES) was noted for replication of the ES by preparatory TLC analysis in the next step.

2.13. Replication of ES by Thin Layer Chromatographic (TLC) Analysis:

EAAC was subjected to TLC analysis several times for replication of ES with the specific Rf value (spots having Rf=0.37 showed a positive result in indirect contact autobiography), where a mixture of petroleum ether and ethanol was used as the mobile phase at a 2:1 ratio. 20×20 cm preparative silica gel plates were used for this purpose.

2.14. Determination of Minimum Inhibitory Concentration (MIC) of ES detected in TLC analysis:

On several replications, ESs were eluted in ethyl acetate in a small beaker and after evaporation *in-vacuo* was dissolved in 1%(V/V) DMSO and subjected to antibacterial assay for determination of MIC for assessing their activities by the same dilution method against all the tested bacteria. Concentrations of active compounds ranged from 1 µg/ml to 20 µg/ml.

2.15. Spraying of specific reagents for phytochemical analyses

ES were sprayed with specific reagents such as Vanillin–sulphuric acid reagent to detect the essential oil components, Dragendorff and Mayer's reagents to detect alkaloid, Liebermann-Burchard reagent to detect saponins and terpenoids, 3% boric acid + 10% oxalic acid to detect flavonoids, and $FeCl_3$ to detect phenols and tannins (Ghosh *et al*, 2008)[25] and Mehta *et al* (2017)[26].

2.16. GC-MS analysis of ES:

ES detected in TLC analysis of EAAC was subjected to GC-MS analysis. The analysis was conducted at Bose Institute, Kolkata. It was performed using Gas Chromatography (Model- TRACE-GC-ULTRA) and Mass Spectrometry (Model- POLARISQ) by TRWAX column capillary equipped with a high-temperature column (Dimension-5mm 30 × 0.25 mm × 0.25 µm) working with 70eV. The stationary phase was polyethylene glycol. One microlitre of the sample was injected into the column and employed using split mode at a split ratio of 1:20. The injector, as well as detector temperature, was set at 250˚C with a split flow of 20 ml/minute. High pure Helium (99.99%) was used as a carrier gas to maintain a constant flow rate of 1ml/min. The column oven temperature was initially kept at 60°C for 2 minutes, and then at 100°C with a ramp of 10°C/min for 3 minutes. Next, the temperature was set at 270°C with a ramp of 5°C /minute for 10 minutes. The maximum temperature was set at 350°C. The total running time of GC was 53 minutes. Identification of compounds was done by matching their recorded spectra with the data bank mass spectra of the National Institute of Standard Technologies (NIST) library provided with the instrument.

2.17. Statistical analysis:

The experimental results were presented by means ± standard deviation (SD) of three replicates worked out in MS-Excel 2007. Data were statistically justified at a 5% level.

3. Results and Discussion:

Results of agar well diffusion assay and antibiotic susceptibility of commonly used antibiotics are depicted in Table 1. Values of MIC, MBC, and MBC/MIC ratio of different extracts are presented in Table 2, which indicated that the MBC/MIC value of EAAC for each bacterium was below 2 advocating their efficacies as bactericides. Phytochemical analysis of EAAC disclosed the presence of alkaloids, saponins, steroids, terpenoids, and the absence of flavonoids, tannins, and cardiac glycosides (Table 3). Corresponding peaks detected in the IR spectrum of EAAC included alcohols with OH and C-O bonds at wave range of 3300-3280 cm^{-1} (hydrogen-bonded, broad peak) and 1100-1000 cm^{-1} stretching respectively. CH bond at 2900-2695 cm^{-1} (CH stretching in Fermi resonance with the overtone of 1390 CH rocking) and CH bond at 1392-1388 cm^{-1} and 980-780 cm^{-1} with rocking, and deformation modes along with the C=O bond at wave range of 1740-1720 cm^{-1} (stretching saturated aliphatic aldehyde) indicated the presence of aldehydes. The presence of ketones [ester containing gamma diketone, (C-(C=O)-CH2-O(C=O)-C)] was supported by the detection of OC=O bond at 1760-1745 cm^{-1} and C=O bond at 1745-1725 cm^{-1} in stretching modes. Esters were depicted by the occurrence of C=O and C-O-C bonds at wave ranges of 1750-1730 and 1300-1100 cm^{-1} respectively. Imides were corresponding to NH, C=O, CNH, and CN bonds at 3280-3200, 1740-1730, 1510-1500 (deformation amide II), and 1235-1165 (stretching amide III) wherein NH, C=O, and C-N bonds at a wavelength range of 3300-3100, 1750-1650 and 1320-1200 cm^{-1} respectively indicated the presence of amides. The ring at a wavelength range of 1625-1575, 1525-1430, and 965-950 and CH bond at 3079-3010, 1170-1150, 1085-1065, and 1030-1010 cm^{-1} indicated the presence of aromatics and alkanes, which were corresponding to CH bonds at a wavelength of 2999-2977 and 2924-2875 cm^{-1} respectively. Thus the corresponding peaks on the FT-IR spectrum confirmed the presence of alcoholic, aldehyde, ketone, ester, imide, amide, aromatic, and alkane groups in EAAC which might be attributed to the presence of several numbers of Phyto-compounds. In addition, the results of preliminary phytochemical screening supported the fact that alkaloids, saponins, steroids, terpenoids present in EAAC might be responsible for the inhibitory action against the pathogens. TLC analysis of EAAC exhibited effective spots (ES) having an Rf value of 0.37 tested against bacterial strains, which showed remarkable sensitivity as depicted in indirect contact bio-autography test and MIC values of ES against all the strains (Table 4). MICs for ES ranged from 6-10 µg/ml (Table 4). Spraying with specific reagents on ES separated on TLC plates showed the presence of different chemical compounds namely alkaloids, saponins and terpenoids, and also essential oils. The active compounds present in ES (Rf= 0.37) were detected in GC-MS analysis by comparing the peaks in the NIST library. The chromatogram developed in GC-MS analysis suggested that four major compounds were present in ES contributing to its antibacterial potency. Bioactive compounds included a naphthalene derivative, 2-isopropyl- naphthalene, an alkaloid, 1,4,4-trimethyl-2,6-piperidinedione, a saponin, 2,4-ditert-butylphenyl -5-hydroxypentanoate and a ketone, 1-hexyl-1-nitrocyclohexane with the retention time of 21.90, 22.58, 23.21, and 20.61 minutes respectively (Figure 1, Figure 2 and Table 5).

The presence of different kinds of phytochemicals in the plant extracts is of great importance due to their therapeutic values. Several authors have evaluated and reported various activities of *A. chama* fruits [15-16] except its antibacterial property, which has been dealt with in this piece of work. In this study, crude extract of *A. chama* fruits (100%) is effective against all the strains with a maximum inhibition against *E. coli* producing an

inhibition zone of 22.00 ± 0.00 mm. We find that ethyl acetate extract of *A. chama* fruits is the most effective amongst the solvent extracts used with the highest inhibitory efficacy with the zone of inhibition of 45.50 ± 0.50 mm against *P. aeruginosa* and lowest inhibitory effect with the zone of inhibition of 37.83 ± 0.58 mm against *S. aureus*. Moreover, the inhibition zone ranges from 18.6 ± 0.58 mm to 37.33 ± 1.15 mm in the case of acetone extract and 11.00 ±0.00 mm to 19.00 ±0.00 mm in the case of petroleum ether extract. The petroleum ether extract is only ineffective against three bacteria namely, *P. aeruginosa*, *B. mycoides*, and *P. flurorescens* (Table 1). Thus, differences are observed between antibacterial activities of the extracts and these differences may be due to the differences in the chemical composition of the extracts as stated by Dhawan and Gupta[27]. On the other hand, antibiotics understudy show comparatively less efficacy than the extracts used, which implies that these extracts are proved to be good candidates for the development of alternatives to these antibiotics (Table 1).

In literature, naphthalene derivatives, alkaloids, saponins, and ketone compounds are reported to have antimicrobial activities [28-31]. Among the compounds detected in GC-MS analysis, 1-hexyl-1-nitrocyclohexane is the most abundant (with an area of a percentage of 43.77%) among the identified compounds and is also known to have antimicrobial property[32]. The data of phytochemical test of ES by spraying different reagents also predict the presence of similar compounds detected in GC-MS analysis. So, it can be concluded that these compounds singly or in combination may be responsible for the bactericidal property of ethyl acetate extract of *A. chama* green fruits. Further, through phytochemical and GC-MS analyses it is clear that its antibacterial property is attributed to the presence of several plant secondary metabolites viz., terpenoids, alkaloids, and saponins indicating their potential as a good source of novel drug formulation of plant origin.

The present study reveals that the extracts of *A. chama* fruits can be considered as a potential source of phytochemical based medicine for infections caused by human pathogens namely *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli,* and *Pseudomonas aeruginosa*, and fish pathogens like *P. putida*, *P. fluorescens*, *B. mycoides* and *B. licheniformis*. The results obtained in this study also reveal that antibacterial efficacies of the extracts against both Gram-positive and Gram-negative bacterial strains are indicative of the presence of a broad spectrum of antibacterial compounds in the *A. chama* fruit extracts. However, it is also necessary to conduct toxicity analysis before its use in clinical trials for treating bacterial infections.

Conclusion:

In conclusion, to the best of our knowledge, this is the first-ever report on the antibacterial activity of *A. chama* fruit extracts. Among the tested extracts of *A. chama* fruits, ethyl acetate extract is most potent and possesses several bioactive phytochemicals having quite high antibacterial activity against all the tested strains of Gram-positive and Gram-negative pathogenic bacteria. This study also suggests further *in vivo* experiments and prospective use of *A. chama* fruit extracts in herbal drugs for the treatment of bacterial diseases.

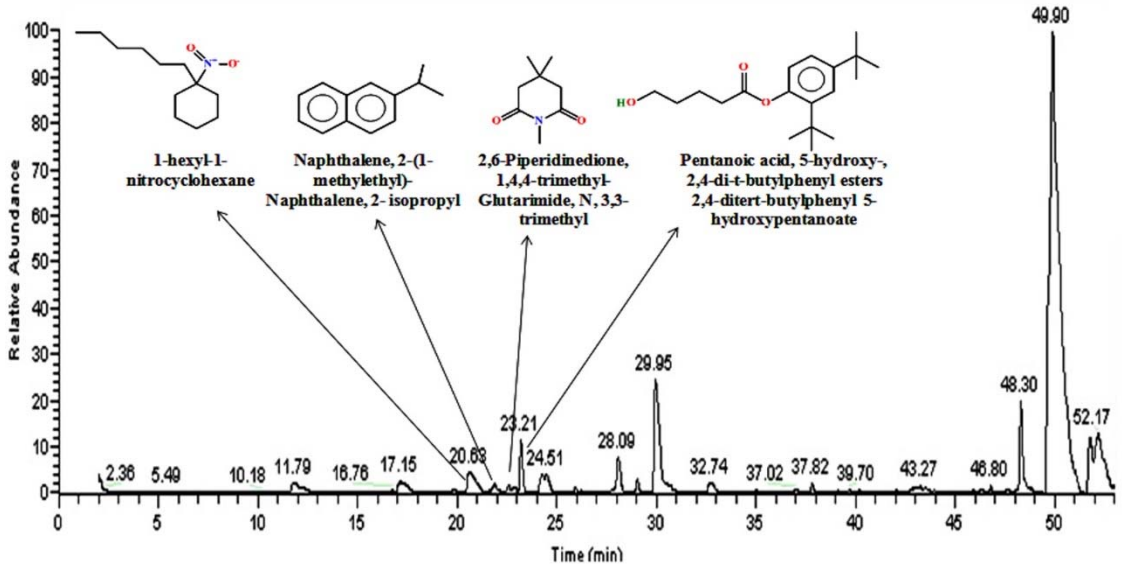
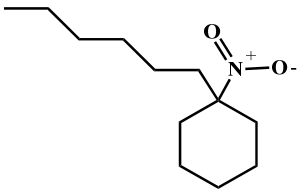
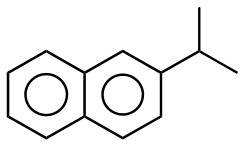


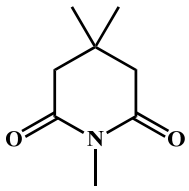
Figure 1: Total Chromatogram of Separated effective spots (ES) (Rf=0.37) of ethyl acetate extract of *Artocarpus chama* fruits (EAAC) by TLC analysis obtained in GC-MS analysis



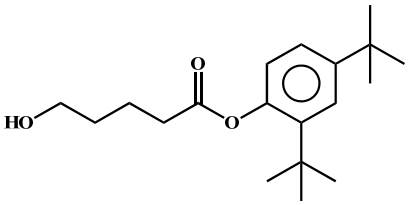
1-hexyl-1-nitrocyclohexane



Naphthalene, 2-(1-methylethyl)-
Naphthalene, 2- isopropyl



2,6-Piperidinedione, 1,4,4-trimethyl-
Glutarimide, N, 3,3-trimethyl



Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters
2,4-ditert-butylphenyl 5-hydroxypentanoate

Figure 2: Chemical compounds detected in Separated effective spots (ES) (Rf=0.37) of ethyl acetate extract of *Artocarpus chama* fruits (EAAC) by TLC analysis

Table 1: Antibacterial effects of different extracts of mature green fruits of *Artocarpus chama* against eight pathogenic bacteria obtained in agar well diffusion assay

Bacteria		Zone of inhibition (mm)* ¥								
	The crude extract (100%)	Petroleum ether extract	Ethyl acetate extract	Acetone extract	Penicillin (10 µg/disc)	Ampicillin (µg/disc)	Kanamicin (5 µg/ disc)	Ciprofloxacin (10 µg/disc)	Sterile distilled water	DMSO (1% v/v)
Human pathogens										
S. aureus MTCC 2940	20.00 ± 0.00	11.00 ±0.00	37.83 ± 0.58	18.6 ± 0.58	Nil	Nil	NA	NA	Nil	Nil
E. coli MTCC 739	22.00 ± 0.00	18.00 ±0.58	41.33 ± 0.76	20.33 ± 0.58	Nil	Nil	14.50 ± 0.87	27.33 ±0.58	Nil	Nil
B. *subtilis* MTCC 441	20.00 ±0.33	12.00 ±0.33	41.00 ± 0.00	21.67 ± 0.58	12.83±0.29	Nil	NA	NA	Nil	Nil
P. *aeruginosa* MTCC 2453	17.50 ±0.33	0.00 ±0.00	45.50 ± 0.50	37.33 ± 1.15	Nil	Nil	15.00 ± 0.00	31.33 ±0.58	Nil	Nil
Fish pathogens										
B. mycoides MTCC 7343	21.50 ± 0.58	0.00 ±0.00	45.33 ± 0.58	27.83 ± 0.76	9.00 ±0.00	Nil	NA	NA	Nil	Nil
B. licheniformis MTCC 503	21.00 ±0.00	15.00 ±0.50	40.33 ± 0.58	19.00 ± 0.00	8.67 ±0.58	Nil	NA	NA	Nil	Nil
P. flurorescens MTCC 103	21.50 ± 0.67	0.00 ±0.00	39.00 ± 0.00	20.33 ± 0.58	Nil	Nil	15.33 ± 0.29	26.83±0.29	Nil	Nil
P. putida MTCC 1654	21.00 ±0.58	19.00 ±0.00	38.33 ± 0.58	19.67 ± 0.58	Nil	Nil	10.33 ± 0.58	29.67 ±0.58	Nil	Nil

*Diameter of well included and diameter of the disc included, ¥ Mean ± SD, NA: Narrow spectrum antibiotics are not applicable for these bacteria

Table 2: Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) of ethyl acetate extract of *Artocarpus chama* fruits (EAAC)

Bacteria	Minimum Inhibitory Concentration (MIC) µg/ml	Minimum Bactericidal Concentration (MBC) µg/ml	MBC/MIC
S. aureus MTCC 2940	250	300	1.20
E. coli MTCC 739	125	200	1.60
B. subtilis MTCC 441	125	200	1.60
P. aeruginosa MTCC 2453	100	175	1.75
B. mycoides MTCC 7343	125	175	1.40
B. licheniformis MTCC 503	250	450	1.80
P. flurorescens MTCC 103	175	250	1.43
P. putida MTCC 1654	250	300	1.20

Table 3: Results of Preliminary phytochemical analysis of ethyl acetate extract of *Artocarpus chama* fruits (EAAC)

Phytochemical test	Result
Flavonoids	-
Tannins	-
Alkaloids	++
Cardiac Glycosides	-
Saponins	++
Steroids	++
Terpenoids	++

Table 4: Minimum Inhibitory Concentrations of Separated effective spots (ES) (Rf=0.37) of Ethyl acetate extract of *Artocarpus chama* fruits (EAAC) by TLC analysis

Bacteria	Minimum Inhibitory Concentration (MIC) µg/ml
S. aureus MTCC 2940	10
E. coli MTCC 739	10
B. subtilis MTCC 441	10
P. aeruginosa MTCC 2453	4
B. mycoides MTCC 7343	8
B. licheniformis MTCC 503	8
P. flurorescens MTCC 103	6
P. putida MTCC 1654	6

Table 5: Details of the compounds detected in GC-MS analysis of Separated effective spots (ES) (Rf=0.37) of ethyl acetate extract of *Artocarpus chama* fruits (EAAC) by TLC analysis

Retention Time	Name of the compound	Molecular formula	Molecular Weight	% Area
20.61	1-hexyl-1-nitrocyclohexane	$C_{12}H_{23}NO_2$	213	43.77
21.90	Naphthalene, 2-(1-methylethyl)- Naphthalene, 2- isopropyl	$C_{13}H_{14}$	170	12.76
22.58	2,6-Piperidinedione, 1,4,4-trimethyl- Glutarimide, N, 3,3-trimethyl	$C_8H_{13}NO_2$	155	5.64
23.21	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters 2,4-ditert-butylphenyl 5-hydroxypentanoate	$C_{90}H_{30}O_3$	306	37.83

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دراسة عن الفعالية المضادة للبكتيريا لمستخلصات مختلفة من ثمار *Artocarpus chama* وتحديد المركبات النشطة بيولوجيًا في أقوى المستخلصات

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ملخص

صممت هذه الدراسة لتقييم القدرة المضادة للبكتيريا لمستخلصات مختلفة من الثمار الخضراء الناضجة من *Artocarpus chama* مع تحديد المركبات النشطة بيولوجيا .تم اختبار الفعالية المضادة للبكتيريا ضد ثمانية أنواع من البكتيريا المسببة للأمراض باستخدام المضادات الحيوية القياسية كعنصر تحكم إيجابي .تم إجراء اختبار حيوي مضاد للبكتيريا عن طريق قياس منطقة التثبيط ، والتركيزات المثبطة الدنيا (MIC) ، والتركيزات الدنيا للجراثيم .(MBC) تم إخضاع المستخلص الأكثر فعالية لاختبارات كيميائية نباتية أولية ، كروماتوجرافيا الطبقة الرقيقة (TLC) ، وتحليل فورييه لتحويل الأشعة تحت الحمراء .تم العثور على مستخلص أسيتات الإيثيل (EAAC)ليكون المستخلص الأكثر فاعلية في إنتاج منطقة تثبيط تبلغ 0.50 ± 45.50 مم ضد *Pseudomonas aeruginosa* MTCC 2453.تعرضت البقع الفعالة (Rf = 0.37) (ES) من EAAC المفصولة عن طريق تحليل TLC للتصوير الذاتي الحيوي للتلامس غير المباشر لتقييم كفاءتها ضد السلالات البكتيرية المختبرة .تراوحت قيم MBC / MIC للمستخلص من 1.20 إلى (2>) 1.80 مما يشير إلى خصائصها القاتلة للجراثيم .تم إجراء التحليل اللوني للغاز - التحليل الطيفي للكتلة ES لتحديد المركبات المضادة للبكتيريا النشطة بيولوجيًا .تم الكشف عن القلويات ، الصابونين ، المنشطات ، التربينويدات ، ومجموعة من المجموعات الوظيفية في .EAAC تراوحت MICs من ES من 10-6 ميكروغرام / مل .تم الكشف عن أربعة مركبات نشطة بيولوجيا في ES في تحليل .GC-MS وهكذا أوضحت هذه الدراسة أن مستخلص أسيتات الإيثيل من ثمار *A. chama* يحتوي على العديد من المركبات النشطة بيولوجيا ذات الخصائص الواعدة للجراثيم ضد السلالات المختبرة من البكتيريا المسببة للأمراض موجبة الجرام وسالبة الجرام.

الكلمات الدالة: *Artocarpus chama*؛ مبيد الجراثيم. الحد الأدنى للتركيز ؛ تحليل FT-IR تحليل GC-MS المركبات النشطة بيولوجيا.

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