Antibacterial efficacy of Jackfruit rag extract against clinically important pathogens and validation of its antimicrobial activity in *Shigella dysenteriae* infected *Drosophila melanogaster* infection model

NV Dhwani[1], Gayathri Raju[1], Sumi E Mathew[1], Gaurav Baranwal[1], Shivakumar B Shivaram[1], Neeraj Katiyar[1], Nilkamal Pramanik[2], Siddharth Jhunjhunwala[2], HB Shilpashree[3], Dinesh A. Nagegowda[3], Ritesh Kumar[4], Ajit K Shasany[4], Raja Biswas[1]* & Sahadev A Shankarappa[1]*

[1]Amrita Center for Nanosciences & Molecular Medicine, Amrita Vishwa Vidyapeetham, Ponekkara P.O., Kochi - 682 041, Kerala, India
[2]Centre for BioSystems Science and Engineering, Indian Institute of Science (IISc), Bengaluru - 560 012, Karnataka, India
[3]Molecular Plant Biology and Biotechnology Lab, CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre, Bengaluru - 560 065, Karnataka, India
[4]Biotechnology Division, CSIR-Central Institute of Medicinal and Aromatic Plants, P.O. CIMAP, Lucknow - 226 015, Uttar Pradesh, India

Exploration of alternative sources of antibacterial compounds is an important and possibly an effective solution to the current challenges in antimicrobial therapy. Plant derived wastes may offer one such alternative. Here, we investigated the antibacterial property of extract derived from a part of the Jackfruit (*Artocarpus heterophyllus* Lam.*)* called 'rag', generally considered as fruit waste. Morpho-physical characterization of the Jackfruit rag extract (JFRE) was performed using Gas-chromatography, where peaks indicative of furfural; pentanoic acid; and hexadecanoic acid were observed. *In vitro* biocompatibility of JFRE was performed using the MTT assay, which showed comparable cellular viability between extract-treated and untreated mouse fibroblast cells. Agar well disc diffusion assay exhibited JFRE induced zones of inhibition for a wide variety of laboratory and clinical strains of Gram-positive and Gram-negative bacteria. Analysis of electron microscope images of bacterial cells suggests that JFRE induces cell death by disintegration of the bacterial cell wall and precipitating intracytoplasmic clumping. The antibacterial activity of the JFREs was further validated *in vivo* using *Shigella dysenteriae* infected fly model, where JFRE pre-fed flies infected with *S. dysenteriae* had significantly reduced mortality compared to controls. JFRE demonstrates broad antibacterial property, both *in vitro* and *in vivo*, possibly by its activity on bacterial cell wall.

Keywords: *Artocarpus heterophyllus*, Gut Microbes, Fruit Fly, Fruit waste

Antimicrobial agents including antiseptics and antibiotics are extensively used for infection control in community and nosocomial settings. Antiseptics, such as chlorhexidine digluconate, triclosan and povidone-iodine are used extensively as surface disinfectants, while antibiotics such as ampicillin, amoxicillin, imipenem and many others are commonly used to treat infections in humans and veterinary practice. Though antimicrobial agents have been a boon to mankind, there are several worrying concerns related to their use. Excessive use of antimicrobial agents worldwide has led to the development of resistant microbes, such as chlorhexidine and colistin resistant *Klebsiella pneumonia*[1], methicillin and linezolid resistant *Staphylococcus aureus*[2], and vancomycin resistant enterococci[3]. Importantly, some of the antiseptics and antibiotics have also been reported to precipitate adverse systemic effects in patients[4]. Povidone-iodine and triclosan has been shown to disrupt thyroid hormone homeostasis[5], while colistin and vancomycin have been associated with renal toxicity[6,7]. Thus, it is imperative that new and safe antimicrobial agents are explored from alternative sources that are generally more user friendly, and yet effective.

Plant extracts have been used for centuries to combat infectious human diseases in different parts of the world. Plant derived medicinal extracts are a

mixture of several compounds, with many extracts reported to have potent antimicrobial activities against wide range of drug resistant microbes[8]. Phenolics, terpenoids, alkaloids and lectins are some of the classes of compounds present in plant extracts that exhibit strong antimicrobial activity. Antimicrobial activity has been reported from various plant extracts, such as *Brillantaisia lamium*[9], *Tribulus terrestris* L.[10], *Oxalis corniculata* L.[11], *Scoparia dulcis* L.[12],*Crinum purpurascens*[13], *Mangifera indica*[14] and *Psidium guajava*[15], against variety of pathogens including *Staphylococcus aureus*, *Enterococcus faecalis*, *Candida tropicalis*, *Cryptococcus neoformans* and *Salmonella* Paratyphi. Thus, plant based materials form an abundant source for antimicrobials that could be economical, easy to process, and efficient against drug-resistant microbes. The Jackfruit is one such plant product that fits this description.

The jackfruit (*Artocarpus heterophyllus* Lam., Fam. *Moraceae*) is a considerably large, weighing approximately 4-10 kg, commercially inexpensive, and widely consumed fruit in Southeast Asia and Africa. In addition to its nutritional value, various components of the fruit possess a plethora of medicinal properties[16]. The fruity arils are known to contain a cocktail of phytonutrients, such as carotenoids[17], isoflavones[18], saponins[18] and phenols[19] that are responsible for antioxidative and immunomodulatory properties[16]. In addition, its leaf extract has been reported to possess antibacterial activities against *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium*, *S. enterica*, *Bacillus cereus*, *Enterococcus faecalis* and *Staphylococcus aureus*[20]. Heartwood has antibacterial activities against *B. subtilis*, *Streptococcus mutans*, *S. pyogenes*, *Staphylococcus aureus* and *S. epidermidis*[21]. In addition, extract derived from jackfruit seeds and shell powder have also been reported to demonstrate antibacterial activities against *L. monocytogenes*[22].

While parts of the ripe jackfruit including the pulpy aril and seeds are a culinary delicacy, the rough, fibrous appendage called 'rag' that make up 10-20% of the fruit are either discarded as non-edible fruit waste, or in some cultures, cooked and consumed. There are no known reports of rag's being used for medicinal purposes but for our earlier reported use of the Jackfruit rag extract (JFRE) as a photo-sensitizer in solar cells[23]. Since rags make up large parts of the fruit and considering the existing lack of clarity towards their use for human benefit, we set out to explore possible medicinal value of the rag, with specific focus on its potential as an alternative to antibiotics against human pathogenic bacteria.

Materials and Methods
Reagents
Methanol, 2-Propanol and hydrochloric acid (HCl) were purchased from Merck, India. The 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), 3-(4,5,dimethyl thiazol2-yl)-2,5-diphenyltetrazolium bromide (MTT), Glutaraldehyde and Triton-X 100 were purchased from Sigma Aldrich, USA. Dulbecco's Modified Eagle's medium (DMEM) and RPMI-1640 were purchased from Lonza, USA and fetal bovine serum (FBS) from Gibco, USA. Luria-Bertani (LB) and De Man, Rogosa and Sharpe (MRS) medium (broth and agar) were purchased from Himedia, India. Mouse dermal fibroblast (L929) cell line was obtained from National Center for Cell Science (NCCS), Pune. All experiments were performed in triplicate unless mentioned otherwise.

Plant material and extract preparation
Rags were separated from the jackfruit obtained from a marked tree located in Shimoga district, Karnataka, India, and stored at −20°C until further use. The collected plant material was identified by Dr. J Jameson, Associate Professor, St. Albert's College, Department of Botany, Ernakulam, Kerala, India, as *Artocarpus heterophyllus* Lam. - Moraceae, and voucher specimens were deposited at St. Albert's College herbarium with voucher number 475.

Air dried rags were homogenized by grinding. Jackfruit rag extract (JFRE) was prepared by suspending the powdered rag (10% w/v) in 80% acidified (1.2 mol/L HCl) methanol and heated at 50°C for 5 h, followed by addition of 100% methanol (1:2). Resulting solution was centrifuged (10,000×g, for 5 min at 4°C), and the supernatant was further concentrated using solvent evaporation techniques and lyophilized. The dried extract was then made up to a final concentration of 100 mg/mL in 100% methanol.

Bacterial and fungal strains
All the bacterial strains including *S. aureus* (SA113), *Mycobacterium smegmatis* (mc[2]155; ATCC700084), *E. coli* (ATCC25922) and *P. aeruginosa* (PA01; ATCC 15692), *Shigella dysenteriae*, *Klebsiella pneumoniae*, *Lactobacillus fermentum*, *Proteus vulgaris*, *Salmonella* Typhi and *Salmonella* Paratyphi A (gifted by Dr. Anil Kumar, Department of Microbiology, Amrita Institute

of Medical Sciences and Research Center, Kochi, India) were cultured in LB broth at 37°C. *Candida albicans* (ATCC 2091) was cultured in Sabouraud dextrose (SD agar) at 37°C.

Morpho-physical characterization of the jackfruit rag and its extract

The surface morphology of rags was analyzed using a scanning electron microscope (SEM) (JEOL, JSM-6490LA, Japan). Images were obtained after sputter coating samples with gold and imaging at an accelerating voltage of 15 kV. Elemental analysis of Jackfruit rag was performed using SEM equipped energy dispersive X-ray (ULTRA55/GEMINI, Zeiss, Germany), after drop casting the rag powder on silicon wafer. Absorbance intensity of JFRE samples were measured from 400 to 700 nm wavelength using a UV-Visible spectrophotometer (Synergy H1, Biotek, USA). Fourier transform infrared (FTIR) spectroscopy was performed on JFRE (Perkin Elmer spectrometer, L1860121, USA), by scanning samples from 4000-500 cm^{-1} for 32 consecutive scans at 27°C. Raman spectroscopy was performed on JFRE using LabRAM HR UV-VIS-NIR Raman microscope, Laser: 785 nm, Filter: D 0.6, Gratings: 600 IR, in the scanning range: 500-3000 cm^{-1}.

Phytochemical tests on JFRE for detection of alkaloids, flavonoids, tannins, saponins and cardiac glycosides[24] was performed according to previously published standard procedures[25]. Gas chromatography-mass spectrometry (GC-MS) quantification of methanolic RAG extract was carried out based on a previously published method[26], using Agilent 7890 GC system equipped with auto-sampler, HP-5ms column and 7977A MSD mass detector (Agilent Technology). Briefly, 2 µL of diluted (1:100) sample (in pentane) was injected in splitless mode with Helium (He) as carrier gas. Inlet temperature was kept at 250°C and the column flow was set for 1.5 mL/min. The column oven was programmed for initial hold of 2 min at 40°C followed by 150°C with the ramp of 5°C per min. After 3 min hold at 150°C, the temperature was raised to 250°C with ramp of 5°C per min and 3 min hold at 250°C. Finally, the oven temperature was raised to 300°C with ramp of 10°C per min and 5 min final hold at 300°C. MSD transfer line, MS Quad, and MS source temperature were kept at 280, 150 and 230°C, respectively. Spectra acquired in scan mode were processed analyzed and annotated using Mass hunter workstation software (Agilent Technology) and NIST 11 library.

Cell viability assay

L929 mouse fibroblasts were seeded in a 96-well plate at a density of 1×10^5 cells per well and cultured under 5% CO_2, at 37°C for 24 h in DMEM media supplemented with 10% FBS and 1% penicillin-streptomycin. After 24 h, the culture media was replaced with fresh media containing JFRE at concentrations of 0.1, 1, 10 and 100 mg/mL. Viability of the JFRE-exposed L929 cells was determined using the MTT assay after 24 h. Solubilized formazan absorbance at 570 nm was measured using Biotek microplate reader (Synergy H1, Biotek, USA)[27].

Hemolysis assay

Female Sprague-Dawley rats weighing around 200-250 g were euthanized using carbon dioxide asphyxiation. Rats used in this study were approved by the Institutional Animal Ethical Committee (IAEC), Amrita Institute of Medical Sciences, Kochi, India in accordance with guidelines set forth by the Committee for Control and Supervision of Experiments on Animals (CPSCSEA). Blood (5 mL) was collected in BD vacutainer tubes and further diluted with PBS (pH 7.4) in 1:10 ratio. Blood samples were centrifuged at 700 g for 5 min to separate erythrocytes from plasma, followed by addition of 0.1,1,10 and 100 mg/mL of JFRE in equal proportion. This mixture was incubated at 37°C for 1 h, followed by centrifugation at 700 g for 5 min. Peak absorbance values of the supernatant was measured at 540 nm (Synergy H1, Biotek, USA). Triton-X 100 (0.2%) treated erythrocyte suspension was used as a comparative positive control, and percent hemolysis in each sample groups was calculated relative to the positive control[28].

Agar well disc diffusion assays

Antimicrobial activity of JFRE was evaluated using standardized agar well-diffusion assay[29,30]. Various microbial strains were plated, and 5 mm wells were created within agar plates. The wells were filled with either, JRFE (0.1, 1,10 and 100 mg/mL), vehicle (80% methanol), or 1% penicillin-streptomycin solution (used as positive control for antibacterial activity assays) or Amphotericin-B (20 mg/mL) used as positive control for antifungal activity assays), and incubated at 37°C for 24-48 h. Plates were imaged using Chemidoc™ Imager (Bio-Rad, USA), and zones of inhibition quantified using ImageJ software[31]. Minimum inhibitory concentration and minimum bactericidal concentration of JFRE against various bacterial strains were determined using the standardized broth dilution method as reported previously[32].

Fly experiments

Adult male *Drosophila melanogaster* flies (4-5 days old) were raised in flasks containing classic banana-agar media, maintained at 60% humidity, and at 28°C[33]. All feeding experiments were performed by placing JFRE (100 mg/mL) or bacterial solution soaked (60 μL) circular filter paper discs on-top of banana-agar feed within each flask. *S. dysenteriae* cultured in 5 mL LB broth (16 h at 37°C), were pelleted and resuspended in PBS and subsequently used to infect flies. Four-hour pre-starved flies were allowed access to *S. dysenteriae* infected feed for 24 h. Flies in the experimental group received JFRE feed, while the control groups received fresh banana agar. Flies were observed for 21 days and the number of surviving flies was counted each day.

Statistical analysis

All data are shown as mean ± standard deviation (SD). Difference in mean values among various experimental groups were statistically tested using one-way ANOVA with post-hoc Bonferroni multiple comparison test, unless mentioned otherwise. *P* value of <0.05 was considered as statistically significant.

Results

Physicochemical characteristics of the jackfruit rag

On gross examination, the JFRs appeared as yellowish white fibrous bands with a smooth and shiny external surface, measuring approximately 5-6 cm in length, 0.5-0.7 cm in width, and weighing about 0.1-0.5 g (Fig. 1A). SEM images of an individual rag exhibited a 'layered' architecture (Fig. 1B), where dense 'honey-comb' patterned plant tissue (Fig. 1C) was interspersed between layers of fibrous sheets. The methanol extract itself was deep red (Fig. 1A, inset) in color and exhibited an absorbance maximum at 540 nm (Fig. 1D). Lyophilization of the extract yielded a dark-reddish powder. Using energy dispersive X-ray spectroscopy, it was determined that the rag extract powder comprised primarily of carbon and oxygen (Table S1. *All supplementary data are available only online along with the respective paper at NOPR repository at http://nopr.res.in*), while other components such as Na, Cl and Si that were observed in the elemental analysis were most likely due to washing techniques and background signal from the silicone sample-loading wafer. Further chemical characterizations were performed using FTIR and Raman spectroscopy. In FTIR (Fig. 2A), the absorption peak at 3375 cm^{-1} likely corresponds to hydroxyl groups (-OH), and 1724 cm^{-1} indicates carbonyl (C=O) corresponding to a carboxyl or ester group arising from pectin or fatty acid residue[34]. The observed, peaks at 1620 cm^{-1} may be due to C=C stretch, and a group of stretching frequencies in the range of 1074-1480 cm^{-1} potentially due to the
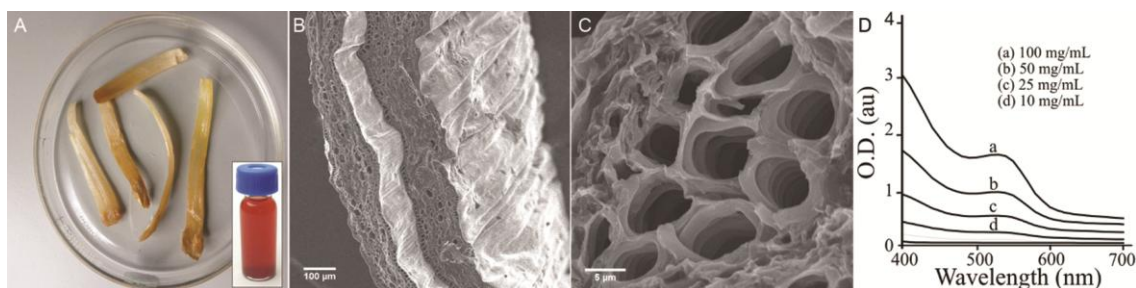

Fig. 1 — Characterization of jackfruit rag and extract. (A) Representative image of a peeled jackfruit exhibiting yellowish-white rags, and (inset) the deep-red methanolic rag extract; (B & C) Representative cross-sectional SEM images of a freshly harvested jackfruit rag; and (D) UV-visible absorption spectra of varying dilutions of JFRE
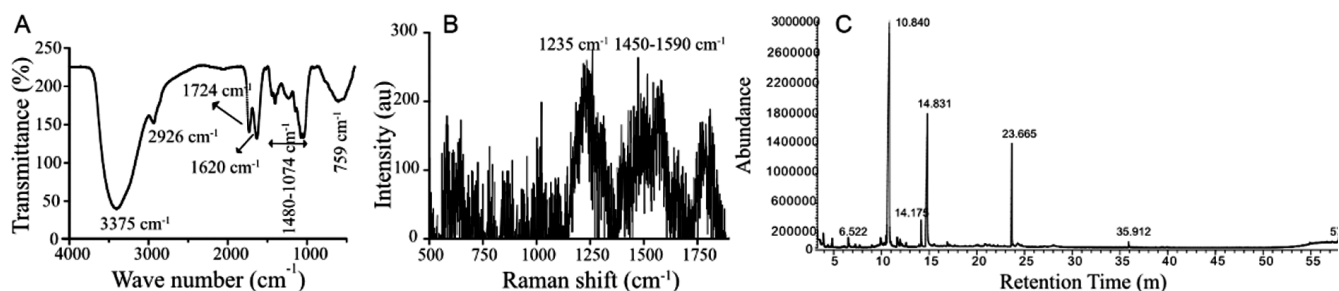

Fig. 2 — Spectroscopic analysis of jackfruit rag extract (JFRE). (A) Fourier Transform Infrared; (B) Raman spectroscopic analyses; and (C) Gas chromatography and mass spectroscopic studies to determine chemical composition of JFRE

	Table 1 — Major constituents present in methanolic RAG extract identified through GC-MS analysis			
RT	Compound name	Molecular formula	Molecular weight (g/mol)	Peak area %
6.52	Furfural	$C_5H_4O_2$	96.021	1.33
10.84	Pentanoic acid, 4-oxo-, methyl ester	$C_6H_{10}O_3$	130.063	53.44
14.17	Levoglucosenone	$C_6H_6O_3$	126.032	2.20
14.82	3-Acetoxy-3-hydroxypropionic acid, methyl ester	$C_6H_{10}O_5$	162.053	27.06
23.66	Citric acid, trimethyl ester	$C_9H_{14}O_7$	234.074	8.91
35.91	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.256	0.47

presence of $-CH_3$ and/or C–O group related to the flavonoids and hydroxyflavonoids[35]. In addition, peaks at 2926 cm^{-1} and 759 cm^{-1}, could be due to the asymmetric stretching of an aliphatic ($-CH_2$) group or bending mode of aromatic (=CH) group. Similarly, the 1450-1590 cm^{-1} bands observed in Raman spectroscopy (Fig. 2B) may be attributed to aliphatic bending vibration and/or aromatic stretching vibration. Additionally, the strong Raman band at 1235 cm^{-1} may be due to the twisting or rocking vibration of $-CH_2$ group present in alicyclic or aliphatic compounds[36].

To further identify the constituent compounds, the methanolic rag extract was subjected to GC-MS analysis. The spectral scan of JFRE annotated through Mass hunter workstation software (Agilent Technology) and NIST 11 library revealed six major constituent peaks which were identified as: furfural; pentanoic acid 4-oxo-methyl ester; levoglucosenone; 3-acetoxy-3-hydroxypropionic acid, methyl ester; citric acid, trimethyl ester; and hexadecanoic acid, methyl ester (Fig. 2C). The major contributing peaks calculated on the basis of peak area abundance were pentanoic acid, 4-oxo-, methyl ester (53.44%), 3-acetoxy-3-hydroxypropionic acid, methyl ester (27.06 %), and citric acid, trimethyl ester (8.91%) (Table 1).

Phytochemical assessment of JFRE

JFRE was subjected to an array of phytochemical screening tests (Supplementary, materials and methods) to determine the presence of common compounds that have been previously recognized to impart medicinal properties in plant extracts[37]. Results from phytochemical screening assays demonstrated the presence of polyphenolics, including anthocyanins, coumarins, and flavonoids. In addition, JFRE tested positive for compounds belonging to terpenoid, saponin and cardiac glycoside groups (Table 2).

***In vitro* biocompatibility testing of JFRE**

To determine possible toxic effects, we examined the response of mouse fibroblast cell-line and rat erythrocytes to an acute exposure of JFRE. Viability of cultured fibroblast cells exposed to media

Table 2 — Biochemical analysis of JFRE	
Class of compounds	Results
Poly-Phenols	+++
Flavonoids	+
Coumarins	++
Anthocyanins	+++
Tannins	-
Terpenoids	++
Alkaloids	-
Proteins	-
Amino acids	-
Saponins	+
Gum and mucilages	-
Cardiotonic glycosides	++
Carbohydrates	+
Reducing sugars	+

(-): Absence of chemical compound; (+): Presence of chemical compound. (+)<(++)<(+++): Based on intensity of characteristic colour

containing 0.1, 1 and 10 mg/mL of JFRE for 24 h was assessed using the MTT assay by measuring formazan absorbance at 570 nm. Interestingly, absorbance measurements from cultures exposed to JFRE were quite similar to those obtained from JFRE-free naïve cells (Fig. 3A), suggesting that the JFRE extract does not affect cellular viability in mammalian cells, at the concentrations tested. MTT measurements in cultures exposed to higher concentrations of JFRE (>10 mg/mL) resulted in unreliable absorbance signals, possibly due to extract induced interference in the assay, and hence higher JFRE concentrations were not tested.

Similarly, blood collected from female Sprague-Dawley rats was processed to isolate erythrocytes, followed by the addition of 0.1, 1, 10 and 100 mg/mL of JFRE. Lysis of erythrocytes was assessed by measuring absorbance at 540 nm of the supernatant separated from the JFRE-erythrocyte mixture. JFRE-induced hemolysis was calculated by normalizing the resulting absorbance value against a completely hemolyzed erythrocyte control. We found that JFRE, at higher concentration induced 28% hemolysis ($P < 0.05$, in comparison to all other groups), while lower concentrations produced hemolysis that was no different than PBS treated controls (Fig. 3B). These results suggest that JFRE possess hemolytic
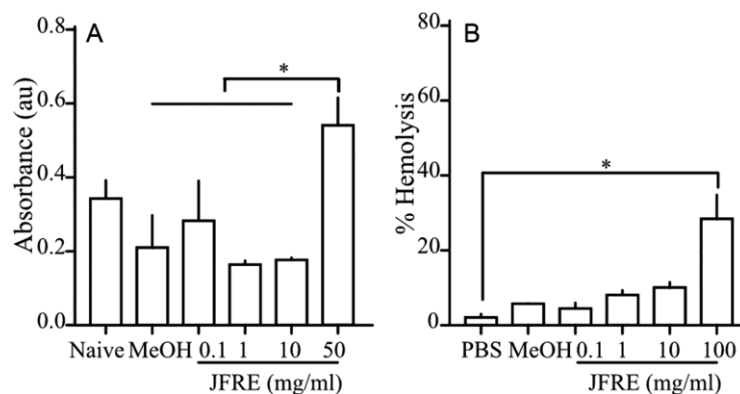
Fig. 3 — *In vitro* biocompatibility profile of the jackfruit rag extract. (A) Quantitative comparison of formazan absorbance values at 570 nm using the MTT assay from L929 cells exposed to varying concentration of JFRE for 24 h; and (B) Quantitative comparison of hemolysis in erythrocyte suspension exposed to varying concentrations of JFRE. [Percent hemolysis was calculated from peak absorbance values at 540 nm normalized to Triton-X induced hemolysis. Data shown are mean ± SD of a representative assay from n=3, performed in triplicate. * indicates P value of <0.05, one-way ANOVA with post-hoc Bonferroni multiple comparison test]
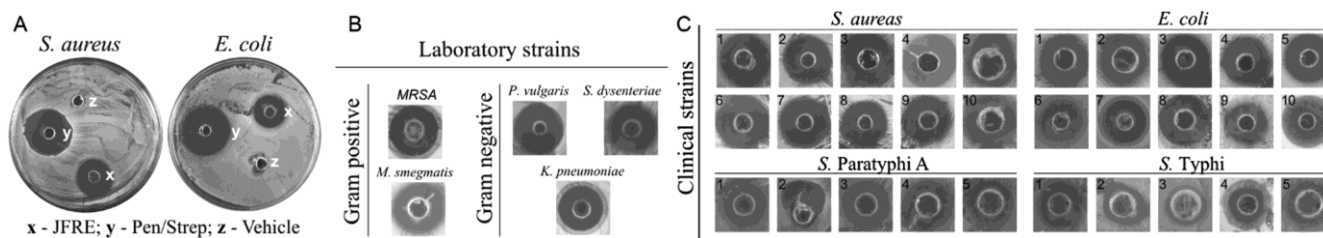

Fig. 4 — *In vitro* antibacterial activity of the jackfruit rag extract. (A) Representative images exhibiting clear bacterial growth inhibitory zones around agar wells containing JFRE (x) and an antibiotic (y) within well-plates that were cultured with laboratory strains of *S. aureus*, and *E. coli*. (B & C) Representative growth inhibitory zones from all laboratory and clinical bacterial strains tested in the study.

properties at higher concentrations, and hence caution needs to be exercised for potential applications related to intravenous use.

Antibacterial property of JFRE

Antimicrobial activity of JFRE was assessed against a wide range of bacterial strains using agar well-diffusion and broth dilution assays. Interestingly, JFRE-exposed culture plates of both Gram-positive and Gram-negative bacteria demonstrated distinct zone of inhibition (Fig. 4A), suggesting broad-spectrum antibacterial activity of JFREs. Further, MIC and MBC values of JFRE against all bacteria tested were in the range of 5-10 and 10-20 mg/mL, respectively (Table 3).

Even though JFRE exhibited inhibition of laboratory bacterial strains (Fig. 4B), it is quite important that similar activity be examined in clinically relevant strains. Hence, we tested anti-bacterial activity of JFRE against strains of methicillin resistant *S. aureus*, *E. coli*, *S.* Paratyphi A and *S.* Typhi (Fig. 4C) derived from patient samples. Zones of inhibition similar to those observed in the laboratory strains were noticed. Since JFRE inhibited bacteria,

Table 3 — Antimicrobial activity of JFRE against laboratory strains of bacteria

Bacteria	MIC (mg/mL)	MBC (mg/mL)
Gram positive		
Staphylococcus aureas	5.0	10
Lactobacillus fermentum	7.5	15
Mycobacterium smegmatis	7.5	15
Gram negative		
Escherichia coli	5.0	10
Shigella dysenteriae	5.0	10
Pseudomonas aeruginosa	5.0	10
Klebsiella pneumoniae	10.0	20
Proteus vulgaris	7.5	15
Salmonella Paratyphi A	7.5	15

irrespective of their strain, source, or antibiotic resistance, it is likely that the mechanism of action is different from conventional antibiotics[37,38]. Incidentally, JFRE did not appear to have antifungal effects against *C. albicans* (data not shown). Further, we imaged JFRE exposed cultures of *S. aureus* and *S. dysenteriae* using SEM. While vehicle treated bacteria exhibited symmetrical cells with regular borders and uniform morphology (Fig. 5A i, iii), JFRE treated bacteria appeared shrunken with irregular margins (Fig. 5A ii, iv). In addition, JFRE components
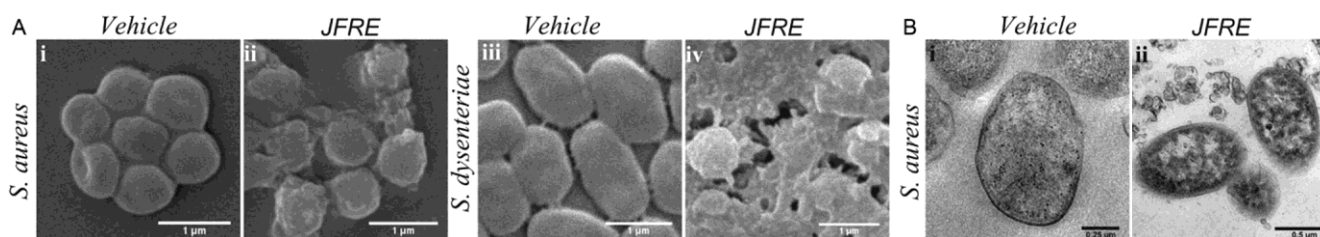
Fig. 5 — Electron microscope images of JFRE treated bacterial cells. Representative (A) SEM images of (i, iii) vehicle; and (ii & iv) JFRE treated *Staphylococcus aureus* and *Shigella dysenteriae*. JFRE treated bacteria exhibited irregular margins and had a 'matted' appearance. (B) TEM imaging of (ii) JFRE treated *Staphylococcus aureus* showed loss of cell wall integrity [magnified segment from Fig. B(ii), inset] and dense cytoplasmic granularity, that was absent in (i) vehicle treated controls.
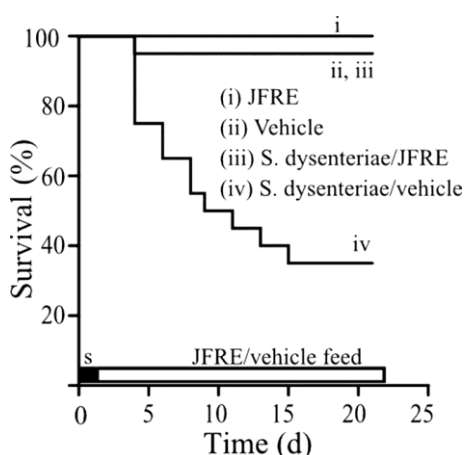

Fig. 6 — Survival of *Shigella dysenteriae* infected flies on JFRE treatment. [Kaplan-Meier survival curves of *S. dysenteriae* pre-fed flies that were either fed JFRE, or vehicle alone. The dark bar on the x-axis (s) indicates the time duration of *S. dysenteriae* feed, while the lighter bar indicates the duration of JFRE/vehicle feed. n=20 per group]

appeared to have covered the bacterial cell surface, giving a matted appearance. Additionally, TEM images clearly showed the disintegration of bacterial surface, with formation of translucent intracellular clumped structures in JFRE treated *S. aureus* (Fig. 5B ii). These results suggest that JFRE could be producing its antimicrobial effects by direct activity on the outer bacterial cell wall or membrane.

Antibacterial effect of JFRE in *S. dysenteriae*-infected flies

To test the applicability of antibacterial property of JFRE *in vivo*, we challenged *S. dysenteriae* fed flies with JFRE and measured *D. melanogaster* survival rate for a period of 3 weeks. *S. dysenteriae*-fed flies exhibited a sharp drop (75%) in survival rate, starting 3-4 days after the infected feed (Fig. 6 iv). The survival rate continued to drop for the next 2 weeks and by the end of 15 days, the survival rate was 35%. No further reduction in survival rate was observed after two weeks. In stark contrast, *S. dysenteriae*-infected flies that were fed with the JFRE

demonstrated a survival rate of 95% at the end of three weeks (Fig. 6 iii). These findings were quite similar to survival rates observed in uninfected flies that were treated with JFRE or vehicle alone (Fig. 6 i, ii), suggesting that JFRE protects against *S. dysenteriae* induced death in *D. melanogaster*.

Discussion

In this study, we set out to explore possible antimicrobial properties of JFREs. There have been several studies characterizing various components of the jackfruit[18] and exhaustive lists have been compiled on their multiple uses[39]. To the best of our knowledge, we have not come across previous reports that have specifically focused on the jackfruit rag.

Morpho-physically, the yellowish white rag has an interesting internal architecture, with each individual rag exhibiting an internal honey-combed pattern, along with a hydrophilic surface, considerable mechanical strength (tensile strength of 0.89 ± 0.4 MPa), and slow degradation in water (Fig. S1). While the actual function of the jackfruit rag is not entirely clear, based on the structure and mechanical strength, it appears that it may be primarily used by the plant as a supportive, packing material to protect the fleshy aril and seeds. During the process of extract preparation, rag powder suspended in acidified methanol yielded deep red colour, with an absorption maximum peak at 540 nm. This observation strongly suggested the presence of anthocyanins[40], which was further confirmed using an array of biochemical screening tests. Further, the chemical characterization techniques of FTIR and Raman spectroscopy also suggested mixture of carbon, oxygen and hydrogen containing compounds. Considering that the jackfruit belongs to the mulberry family which are well known for their high polyphenolic and anthocyanin content[41], it would appear that jackfruit rag components also share similar characteristics. Incidentally, GC MS analysis revealed

strong peaks indicating the presence of carboxylic acids, such as pentanoic acid and hydroxypropionic acid, that have been previously recognized to possess antibacterial properties[42-44]. Antioxidative property of JFRE was found to be moderate to low (data not shown) and was not further pursued.

We found that JFRE produced inhibitory zones in bacterial cultures at concentration of around 100 mg/mL (MIC 5-10 mg/mL), while lower concentrations produced weak or no inhibitory zones in culture plates. While inhibitory zones were observed in both Gram-positive and Gram-negative laboratory strains, we were surprised to observe similar zones of inhibition in all the strains of clinically relevant cultures of *S. aureus*, *E. coli*, *S.* Typhi and *S.* Paratyphi A. This wide ranging spectrum of antibacterial activity by JFRE against different bacterial strains, strongly points towards a possible target mechanism that could be common against all bacteria. SEM images clearly showed rag extract induced blebbing of bacterial cells, along with aggregation, and change in shape, possibly due to membrane permeabilization. Previous studies have shown that bacterial cells that are normally impermeable to FITC, a fluorescent dye, become FITC-permeant[45] after exposure to alcoholic extracts of various plant extracts mainly due to membrane permeabilization, destabilization, and disruption of membrane potential, resulting in cell blebbing and leakage of cellular contents[38]. In agreement with previous studies on other plant extracts[46], our results also indicate that JFRE causes changes in bacterial cell morphology that eventually leads to cell death.

In view of these findings, we further tested the antimicrobial activity of JFREs in *S. dysenteriae* infected fly model. The protective effect of rag extract against *S. dysenteriae* induced death was quite apparent, with 95% of the flies surviving for more than 3 weeks. This effect was consistently observed in all the three batches of fly cultures tested at different times. Considering that flies were pre-fed with *S. dysenteriae* infected agar a day before rag extract treatment, the rag extract appears to have directly prevented fly gut infection by eliminating *S. dysenteriae* and protecting flies from *S. dysenteriae* enterotoxin induced death. It is also important to note that crude extracts, such as the one used in this study will contain a mixture of various bioactive molecules

acting in cohesion to bring about a functional outcome, rather than a single active molecule exerting its effect[47].

Conclusion

The above results demonstrate that the Jackfruit rag extract (JFRE) exhibited significant antibacterial activity against both laboratory and clinical strains and prevented *Shigella dysenteriae* induced mortality in *Drosophila melanogaster*. Phytochemical screening assays of the rag extract demonstrated the presence of polyphenolics, including anthocyanins, coumarins and flavonoids, which were likely responsible for the JFRE-induced destabilization of bacterial cell wall. However, further studies are needed to identify and confirm the exact mechanism of JFRE induced gut protection and its role in disrupting *S. dysenteriae* pathogenesis.

Conflict of Interest

Authors declare no competing interests.

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