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Revealing the Antimicrobial Potential of Jackfruit Rag Extract: Profiling with Spectro analysis

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ABSTRACT

Artocarpus heterophyllus Lam, commonly known as jackfruit, exhibits antimicrobial properties, particularly in its latex filaments called "rag." Extracts from jackfruit rag rich in phytonutrients like phenolic compounds, exhibit antimicrobial activity and can serve as alternatives to conventional antimicrobial agents. This study focuses on extracting phytochemicals from jackfruit rag and evaluating antimicrobial effects against foodborne pathogens: *Escherichia coli*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Salmonella*, *Aspergillus niger*, *Candida albicans*, *Penicillium digitatum*. FT-IR spectroscopy identified phenol compounds with an O-H group at 3384.84 cm⁻¹. LC-MS analysis identified phenolic compounds (3-Hydroxybenzylhydrazine), alkaloids (Tryptamine, Myoscorpine), benzoic acid ester (Butyl isobutyl phthalate, Dictamdiol), and three putatively identified compounds. These findings suggest the presence of phenolic compounds known for antimicrobial activities. The purified extract at 1.8 mg/mL showed the strongest inhibition activity, while the petroleum ether extract (S1) significantly inhibited *E. coli* at 0.1125 mg/mL compared to other extracts. However, antifungal activity varied among extracts. This study underscores the potential of rag extracts as cost-effective alternatives to conventional antimicrobials, addressing challenges in antimicrobial therapy, especially against drug-resistant microbes. This study contributes to the applications of plant-derived antimicrobial compounds, highlighting the significance of utilizing plant waste for growing knowledge and potential applications in antimicrobial research.

Keywords: Antifungal activity, Antimicrobial activity, Jack fruit rag, Phytochemicals

INTRODUCTION

In the search for novel antimicrobial agents, the focus turns to the botanical marvel known as *Artocarpus heterophyllus*, commonly referred to as jackfruit, belonging to the Moraceae family. Recognized in Latin America as "Tree Bread," jackfruit consists of various species, including *Artocarpus maxima* Blanco, *Artocarpus heterophylla* Lam, *Artocarpus brasiliensis* Gomez, and *Artocarpus philippinensis* Lam (Gupta et al., 2011). The large fruits, weighing 4.5 to 27.3 kg, undergo an appealing transformation from green in immature to brownish-yellow in ripeness due to the constantly changing interactions of chlorophyll, carotenoid, and anthocyanin pigments. The fruit contains the edible pulp that surrounds each seed. Between the seeds and the edible pulp is the inedible "rag" (Crane et al., 2016). Besides serving as a nutritional powerhouse, jackfruit has therapeutic properties due to its rich composition of carbohydrates, minerals, carboxylic acids, dietary fiber, vitamins, and minerals (Toor et al., 2021). The jackfruit, presenting itself as a plant product aligning with the characteristics of antimicrobials, stands out as an easily processable resource. Traditional medicine uses every part of the jackfruit tree, but the global waste generated exceeds the fruit yield (Dam et al., 2012).

The secondary metabolites of *A. heterophyllus* Lam contain a wide range of phytonutrients such as lignans, flavones, saponins, carotenoids, tannins, prenylflavones, and sterols, which exhibit anti-oxidant, anti-hypertensive, anti-aging, anti-inflammatory, anti-helminthics, anti-microbial, anti-diabetic and anticarcinogenic properties (Ahasan et al., 2021; Khan et al., 2021, Kumari et al., 2020). In the face of diseases caused by phytopathogens, especially those of bacterial and fungal origins, agriculture demands innovative approaches (Harborne, 1998). Many synthetic compounds also possess anti-diabetic properties (Shivanna et al., 2022, Deepika et al., 2022, Prashanth et al., 2021, Sreepathi et al., 2024). Enzymes such as Xanthine oxidase also possesses anti-microbial properties (Jyothi et al., 2022, Sreepathi et al., 2024).

Jackfruit "Rag" (JFR) refers to latex filaments situated outside the jackfruit bulb (Begum et al., 2014) appearing yellowish white fibrous bands measuring 4-6cm in length and 0.5-0.7 cm in width (Biworo et al., 2015), JFR not only serves as a protecting agent for the arils and seeds but also exhibits a significant antimicrobial activity. The cocktail of phytonutrients such as isoflavones, carotenoids, saponins, and phenols sets JFR as an intriguing option for treating or preventing drug-resistant bacterial infections [Swami et al., 2012; García (2018), Meenakshi et al., 2024].

As global pesticide use rises, causing adverse effects on the environment, human health, and insect resistance, the search for environmentally acceptable biocidal agents intensifies (Vinusha et al., 2020). This has accelerated the search for new, ecologically friendly biocidal agents, such as plant extracts, bioactive peptides, probiotics, and essential oils with antioxidant, antibacterial properties, anti-convulsant and anti-hyperglycemic properties [Pietsch and Meakins (1976), Patil et al., 2022, Ramu et al., 2017, Pramila et al., 2022, Patil et al., 2020, Martiz et al., 2022, Ramu et al., 2022, Sreepathi et al., 2022]. The increased use of antimicrobial agents, particularly antiseptics and antibiotics, has resulted in resistant bacteria, prompting a shift towards plant extracts, which have been traditionally used for generations to treat infectious diseases in many regions worldwide (Rios and Recio, 2005; Gupta et al., 2011, Kumar et al., 2022, Meenakshi et al., 2024). The phytoconstituent can be delivered with improved anti-microbial activity (Mahadev et al., 2022). Poor control of diabetes also leads to the formation of biofilms in the oral cavity and leads to gum diseases (B.G. et al., 2024).

Despite the economic and nutritional importance of jackfruit, research gaps continue to exist, particularly concerning its rag component. This study aims to bring attention to the jackfruit rag, which is often neglected. It is investigating the antimicrobial properties of jackfruit rag extract and shedding light on its efficacy as a long-term alternative to antibiotics for combating foodborne pathogens.

Materials and methods

Sample collection:

Jackfruit of *Artocarpus heterophyllus* varieties (n=9) were collected from local farms and markets in and around Mangalore. To prepare the samples for analysis, the rags were methodically separated from the fruits and subsequently subjected to a drying process in a hot air oven at 42 °C until constant weight. Following this, the dried rag was finely powdered using a miller and used for extraction [Ismail and Hong (2002)].

Preparation of extracts:**Petroleum ether extract:**

JFRE of petroleum ether was made by suspending the powdered rag (10 % w/v) and keeping it in a shaker incubator at 27°C, 120 rpm for 48 hours, followed by filtration. After filtration, the filtrate was concentrated using a rotatory evaporator at 40°C, 40 rpm. The resultant residue was then subjected to drying at room temperature (RT) for further extraction processes.

Chloroform extract:

Dried residue obtained from the petroleum ether extract was suspended in Chloroform (10 % w/v) and kept in a shaker incubator at 27°C, 120 rpm for 48 hours followed by filtration. After filtration, the filtrate was concentrated using a rotatory evaporator by solvent evaporation technique at 40°C, 40 rpm. The obtained residue was then subjected to drying at RT for further extraction processes.

Ethanol extract:

JFRE obtained from chloroform extract were completely dried and suspended in (10% w/v) ethanol, which was kept in a shaker incubator at 27 °C, 120 rpm for 48 hours, followed by filtration. The filtrate was concentrated through solvent evaporation using a rotatory evaporator at 40°C and 40 rpm. The residue was then dried at room temperature for further extraction.

Aqueous extract:

The dried powdered rag obtained after ethanol extract was suspended in (10% w/v) distilled water and kept in a shaker incubator at 27 °C, 120 rpm for 48 hours followed by filtration. The obtained residue was allowed to dry at room temperature, while the filtrate was concentrated further using solvent evaporation techniques via lyophilization, with the resulting residue also allowed to dry.

Antibacterial activity:**Agar well disc diffusion assay**

The antimicrobial efficacy of JFRE was assessed using a standardized agar well-diffusion assay (Purkayastha and Dahiya, 2012, Kumari et al., 2022, Divyashree et al., 2024). Foodborne pathogens like *Staphylococcus aureus* (ATCC-23235), *Escherichia coli* (ATCC-25922), *Salmonella enterica* (ATCC-25928), *Vibrio parahaemolyticus* (ATCC-17802) were cultured on Muller Hinton agar (MHA). 10µl of extracts were added to the disc and kept for evaporation. After evaporation, the disc was placed on sterile MHA plates previously inoculated with a fresh cell suspension of the tested bacteria. The plates were then incubated overnight at 37°C. Antimicrobial activity was assessed by measuring the growth inhibition zone diameter around the disc in mm and the microbroth dilution method was employed to determine the minimum inhibitory concentration (MIC).

Minimum Inhibitory Concentration (MIC):

Cultures were inoculated to Muller Hinton broth (MHB) and kept in a shaker incubator until the growth reached 0.3-0.6 OD. MIC was performed in a 96-well plate. 95 µL of MHB with dilutions of 1:1 was added to each well. 100 µL of the purified extract was taken as neat ranging from 1800 µg/mL were added to the first well and then diluted till the last well and 100µL of the mixture was discarded from the 10th well. The extract was mixed thoroughly by pipetting after each dilution. Subsequently, 5 µL of cultures were added, and MHB alone served as control. The

plate was incubated at 37°C for 18-24 hours, and the MIC was determined as the concentration with no visible growth after incubation [Purkayastha and Dahiya (2012), Pramila et al., 2020, Kokila et al., 2022, Huligere et al., 2023].

Antifungal activity:

Antifungal susceptibility was measured using the disc diffusion method, using strains of *Penicillium digitatum* (ATCC-10030), *Aspergillus niger* (ATCC-6275), and *Candida albicans* (ATCC-76485). The phytopathogens were cultured on potato dextrose agar medium (PDA) at 37°C ± 2 °C for 3 days. Subsequently, sterile paper discs impregnated with solvent extracts were placed on an agar plate. Inhibition zones were determined following a 48-hour incubation at 30°C. The experiments were repeated in duplicate, and mean values were calculated from the results [Cabrera and Montenegro (2013)].

Characterization of phytochemicals

The analysis of phytochemicals in jackfruit rag was done using FTIR and LC-MS spectroscopy technique, which provides significant functional and structural data derived from examining the photosystems data.

Fourier Transform Infrared Spectroscopy

FTIR spectroscopy was performed for each sample to characterize the type of compound. When the sample is subjected to FTIR analysis, a series of spectra are produced due to vibrations caused by the IR radiations. As the IR radiation is passed through the sample, based on the surface functional groups a disturbance is produced resulting in sharp peaks in the spectrum.

Liquid chromatography- Mass spectrometry conditions

LC-MS was used to analyze the phytochemical profile of sample DE5, which exhibited antibacterial activity using the (Tanmay et al., 2020) procedure.

The analysis was carried out by using an Acquity Ultra Performance Liquid Chromatograph (UPLC) with QToF-MS (Xevo QToF, Waters, USA), operating under electrospray ionization (ESI) with a mass resolution of 20,000, controlled by UNIFI software (version 1.7, Waters Corporation). The setup allowed rapid switching between low energy (4 V, full scan MS) and high energy (10–60 V ramping) scans in a single LC run, providing intact molecular and fragment ion data. The parameters included were capillary voltage at 3 kV, sampling cone at 30 V, extraction cone at 5 V, source temperature at 120°C, desolvation temperature at 500°C, desolvation gas flow at 1000 L/h, and cone gas flow at 50 L/h. The calibration was done using 0.5 mM sodium formate, and mass correction utilized leucine enkephalin (m/z 556.2771 in positive and 554.2670 in negative polarity) at 10 μ L/min and 2 μ g/mL concentration every 20 seconds. Chromatographic separation occurred on an Acquity UPLC BEH C18 column (2.1 $\times$ 100 mm, 1.8 μ m, Waters India Pvt. Ltd., Bengaluru) at 35°C. The mobile phase consisted of A: methanol (10:90, v/v) and B: methanol (90:10, v/v) with 0.1% formic acid, using a gradient program at a flow rate of 0.4 mL/min (0-0.5 min/90% A, 4.5 min/50% A, 4.5-8 min/50-2% A, 8-11 min/2% A, 11-11.5 min/2-90% A, 12-15 min/90% A).

RESULTS AND DISCUSSION

RESULTS

Sample preparation

The separated rag presented itself as yellowish-white fibrous bands with a glossy and sleek outer surface, and it was measured around 5-6 cm in length, 0.5-0.7 cm in width, and weighed about 0.1-0.5 g (**Figure 1a**). These rags were subjected to drying under aseptic conditions resulting in a yellow appearance (**Figure 1b**). Subsequently, the dried rag was finely powdered using a miller under sterile conditions. A 20 g of the powdered rag was subjected to extraction methods. The sample was prepared by dissolving in a 1:1 ratio (1 gm of sample in 1 mL of distilled water).

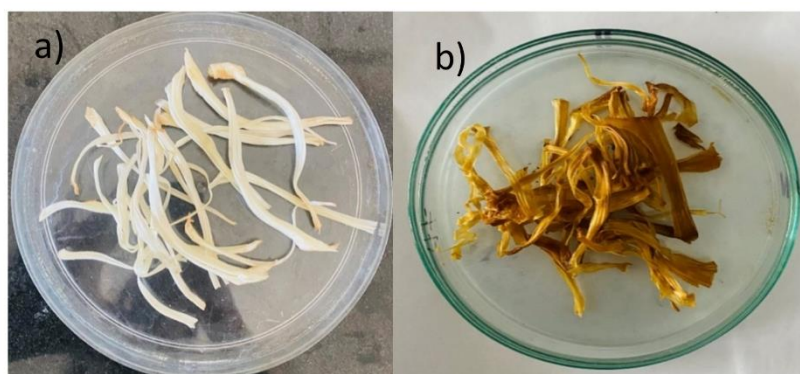


Figure 1. Representative images of rag samples **a)** before drying and **b)** after drying

Preparation of extracts:

In the petroleum ether extraction method, it was observed that S2 had the highest yield of extract, weighing 7.4 g, followed by PE1, weighing 5.8 g. In contrast, the lowest extract was observed in PE4 weighing 2.2 g. The dried residue obtained from the petroleum ether extract method was subjected to the chloroform extraction method using a rotary evaporator. During this process, it was observed that S1 yielded the highest extract weighing 4.8 g, followed by CE5 with a yield of 4.1 g. Conversely, the least-yield extract was obtained in CE4 at about 1.6 g. The residue was dried and subjected to ethanol extraction using rotary evaporation after chloroform extraction. It was observed that EE6 yielded the highest extract, weighing 3.5 g, followed by EE2, showing 3.2 g. The extract with the least yield was obtained in S9. After ethanol extraction, the residue was dried and subjected to distilled water extraction using a lyophilizer. It was observed that DE1 yielded the highest extract followed by S4. The extract with the lowest yield was obtained in DE7 (**Table 1, Figure 2**).

Table 1. Amount of petroleum ether extract obtained from different samples

SI.No	Samples	Locations	JFRE Extract code	Obtained extract (g)

1	Sample 1 (S1)	Lat:12.762300°, Long:75.200260 °	PE1	5.8
			CE1	4.8
			EE1	3.0
			DE1	2.8
2	Sample 2 (S2)	Lat:12.837140°, Long:75.254227°	PE2	7.4
			CE2	3.1
			EE2	3.2
			DE2	1.9
3	Sample 3 (S3)	Long:12.236310°, Long:75.850780°	PE3	4.1
			CE3	2.9
			EE3	2.8
			DE3	1.8
4	Sample 4 (S4)	Lat:12.520160°, Long:78.223450°	PE4	2.2
			CE4	1.6
			EE4	2.4
			DE4	2.2
5	Sample 5 (S5)	Lat:12.878200°, Long:75.034000 °	PE5	3.1
			CE5	4.1
			EE5	3.1
			DE5	2.1
6	Sample 6 (S6)	Lat:12.873720°, Long:74.996670°	PE6	2.3
			CE6	3.8
			EE6	3.5
			DE6	2.3
7	Sample 7 (S7)	Lat:12.236310°, Long:75.850780°	PE7	3.7
			CE7	3.6
			EE7	2.7

			DE7	1.7
8	Sample 8 (S8)	Lat:12.800393°, Long:74.885357°	PE8	4.2
			CE8	3.1
			EE8	1.9
			DE8	2.2
9	Sample 9 (S9)	Lat:12.800395°, Long:74.885363°	PE9	2.7
			CE9	1.7
			EE9	1.8
			DE9	1.7

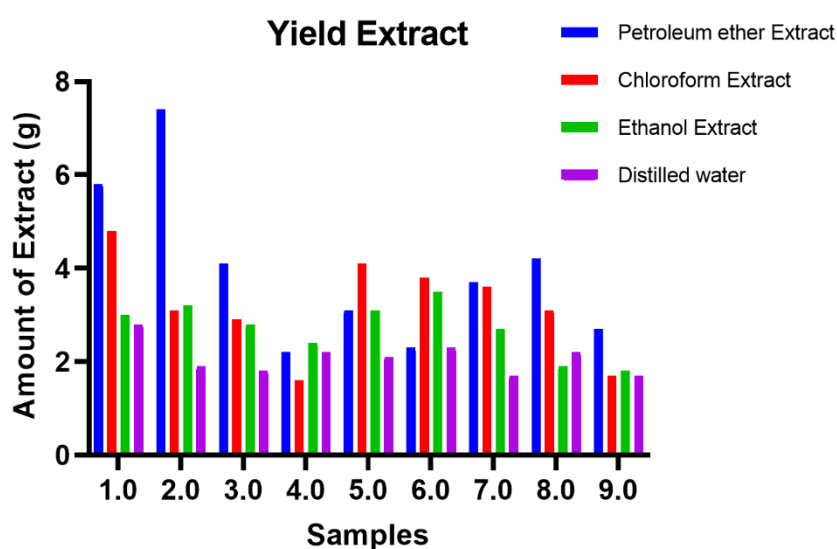


Figure 2. Amount of extract obtained from each sample for different extraction methods.

Antimicrobial activity of JFRE

MIC: minimum inhibition concentration for JFRE

The investigation involved determining the MIC using a two-fold dilution series for five extracts. Specifically, Petroleum and chloroform extract of sample 1 showed inhibitory effects against *E. coli* at a concentration of 0.1125 µg/mL, CE1 demonstrated inhibitory effects against *E. coli* concentration at 0.225 µg/mL, EE5

showed against *Vibrio parahaemolyticus* concentration at 0.225 $\mu\text{g/mL}$, EE7 demonstrated inhibitory effects against *E. coli* at 0.4 mg/mL, DE5 showed inhibitory effects against *Salmonella* at 0.225 $\mu\text{g/mL}$ (**Figure 3**).

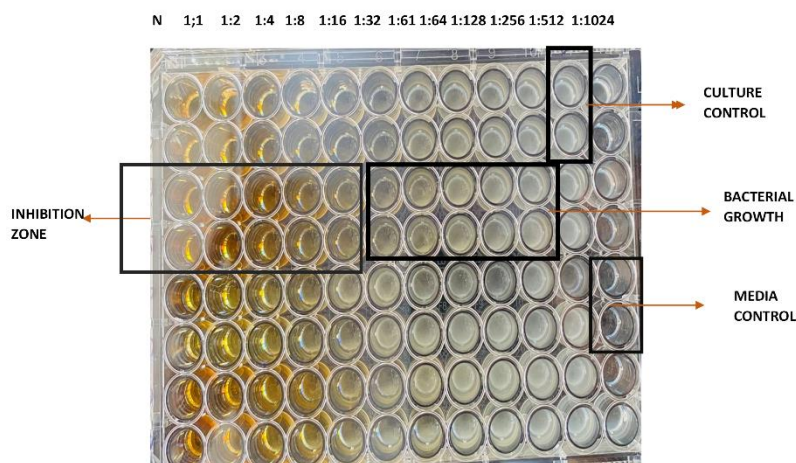


Figure 3. Representative image of MIC concentration of rag extract against food-borne pathogens

Antifungal activity

The absence of antifungal activity in the extracts from all samples can be related to the non-presence of the component responsible for antifungal effects across all extracts.

Phytochemical characterization of the jackfruit rag

FT-IR measurements were carried out to identify the major functional groups in the analysed sample. The extracted sample was examined using FT-IR spectroscopy, which showed the presence of MIC. During the FTIR analysis, the sample generated a series of spectra in response to the vibrations caused by the IR radiations. As the IR radiation traversed through the sample, based on the surface functional groups a disturbance was produced resulting in sharp peaks in the spectrum. (**Table 2**).

Table 2. FT-IR peak values and functional groups in JFRE

Sl. No.	Frequency ranges (cm ⁻¹)	Frequency peak values (cm ⁻¹)	Vibration/bond	Specific functional group	Chemical compound
1	3600–3200	3525.94	O–H stretch	Alcohols, phenols (hydrogen bonding)	Aromatic
2	3400–3250	3728.21	N–H stretch	1°, 2° amines and amides	Amines and amides
3	3000–2850	2981.95	C–H stretch	Alkanes	Aliphatic
4	1680–1620	1651.38	C=C stretch	Alkene	Aliphatic
5	1320–1000	1258.59	C–O stretch	Alcohols, carboxylic acids, esters, and ethers	Acid and alcohol
6	1320–1000	1039.70	C–O	Alcohols, carboxylic acids, esters, and ethers	Acid and alcohol

Analysis of Graphical representation of FTIR spectrum analysis of PE1

The FTIR spectrum analysis of the chloroform extract from sample S1 is depicted, showcasing distinct peaks associated with various functional groups indicative of specific phytochemical compounds. The absorption peak at 3748.72 cm^{-1} indicates the presence of hydroxyl groups (-OH) or the possible presence of alkaloids, potentially indicating the N-H stretch. Additionally, the absorption peak at 1734 cm^{-1} indicates carbonyl (C=O), originating from a carboxyl or ester group from pectin or fatty acid residue. The peaks observed at 818 cm^{-1} , 774 cm^{-1} may be attributable to the C=C stretch. In contrast, a cluster of stretching frequencies ranging from 1080 cm^{-1} to 1455 cm^{-1} could suggest the presence of -CH₃ and C-O groups associated with flavonoids and hydroxy flavonoids. Furthermore, the peaks at 2921 cm^{-1} could result from the asymmetric stretching of an aliphatic (-CH₂) group or the bending mode of the aromatic (=CH) group. (Figure 4).

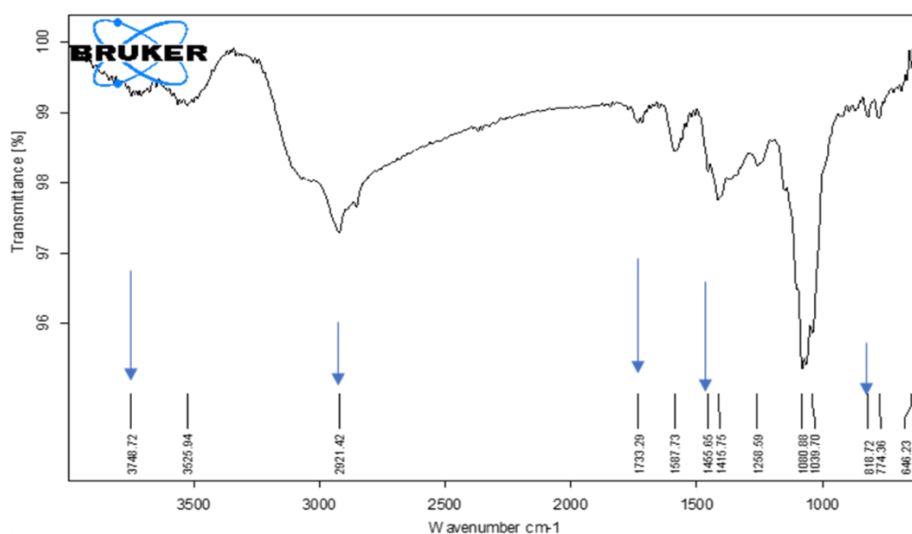


Figure 4. FTIR Spectroscopic analysis of jackfruit rag PE1.

Analysis of Graphical representation of FTIR spectrum analysis of CE1.

A graphical representation of the FTIR spectrum analysis of S1's chloroform extract, with each distinct peak representing a different functional group and indicating specific phytochemical compounds. Arrows in the graphical representation highlight absorption peaks at 3555 cm^{-1} , likely corresponding to hydroxyl groups (-OH).

Another peak at 1732 cm^{-1} indicates a carbonyl group ($\text{C}=\text{O}$), which could be a carboxyl or ester group arising from pectin or fatty acid residue. The peaks observed at 1651 cm^{-1} may be due to $\text{C}=\text{C}$ stretch, while a group of stretching frequencies ranging from 1086 cm^{-1} to 1455 cm^{-1} potentially corresponds to $-\text{CH}_3$ and/or $\text{C}-\text{O}$ group related to the flavonoids and hydroxy flavonoids. Additionally, peaks at 2925 cm^{-1} could be attributed to the asymmetric stretching of an aliphatic ($-\text{CH}_2$) group or the bending mode of the aromatic ($=\text{CH}$) group. (**Figure 5**).

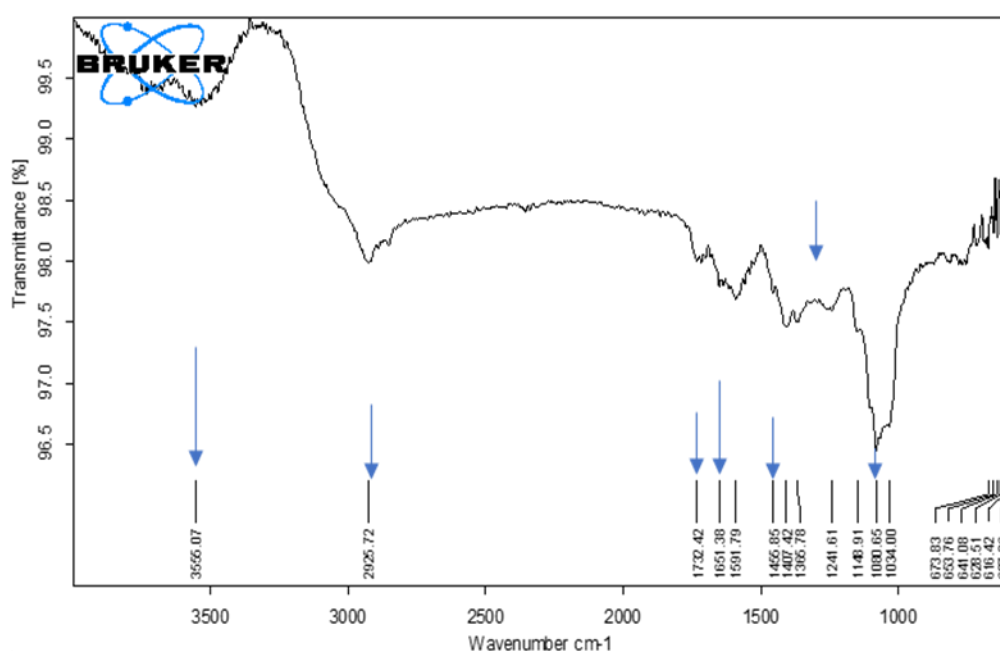


Figure 5. FTIR Spectroscopic analysis of jackfruit rag of CE1

Analysis of Graphical representation of FTIR spectrum analysis of EE5.

A graphical representation of the FTIR spectrum analysis of the S5 ethanol extract, with each distinct peak representing a different functional group and implying the particular phytochemical compound. The absorption peaks at 3711 cm^{-1} and 35181 cm^{-1} likely correspond to hydroxyl groups ($-\text{OH}$), while the peak at 1718 cm^{-1} indicates carbonyl ($\text{C}=\text{O}$) group, suggesting the presence of a carboxyl or ester group arising from pectin or fatty acid residue. The peaks at 1558 cm^{-1} may be due to the

C=C stretch. A range of stretching frequencies between 1081 cm^{-1} and 1455 cm^{-1} potentially indicates the presence of $-\text{CH}_3$ and/or C–O groups related to flavonoids and hydroxy flavonoids. Additionally, the peaks at 2978 cm^{-1} and 2918 cm^{-1} could be attributed to the asymmetric stretching of an aliphatic ($-\text{CH}_2$) group or the bending mode of the aromatic ($=\text{CH}$) group. These observations strongly suggest the presence of phenolic compounds, known for their antimicrobial activities (**Figure 6**)

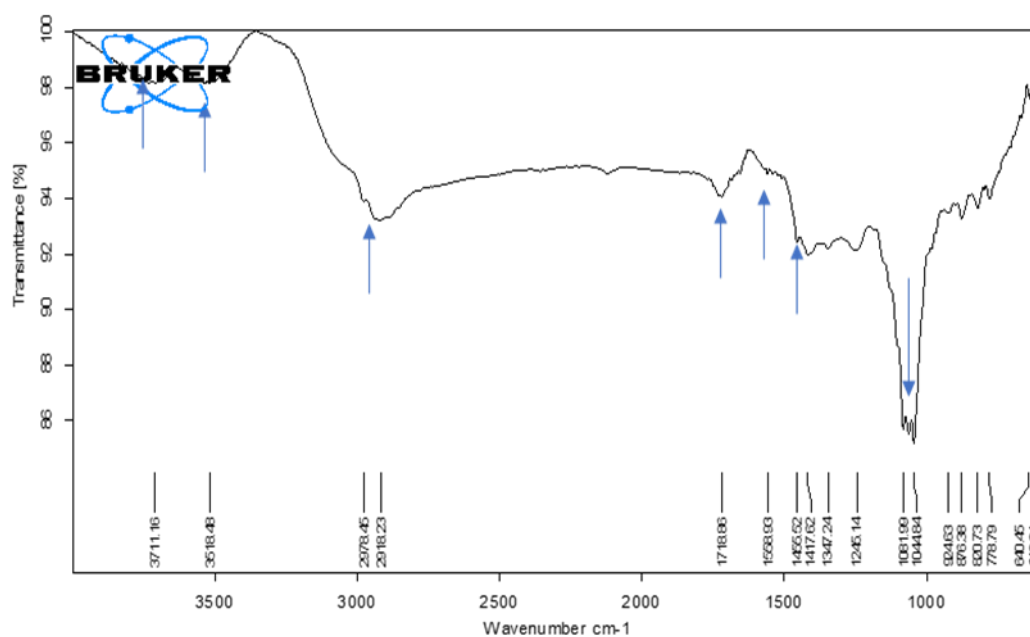


Figure 6. FTIR Spectroscopic analysis of jackfruit rag EE5

Analysis of Graphical representation of FTIR spectrum analysis of EE7.

The FTIR spectrum analysis of the S5 ethanol extract with each distinct peak corresponding to various functional groups and indicating specific phytochemical compounds. The absorption peaks at 3733 cm^{-1} and 3522 cm^{-1} are likely for hydroxyl groups ($-\text{OH}$), while 1717 cm^{-1} indicates a carbonyl ($\text{C}=\text{O}$) group associated with carboxyl or ester group from pectin or fatty acid residues. The peaks observed at 1455 cm^{-1} may be attributed to the C=C stretch. Additionally, a cluster of stretching frequencies between 1081 cm^{-1} and 1416 cm^{-1} may be due to the presence of $-\text{CH}_3$ and/or C–O groups related to the flavonoids and hydroxy flavonoids. The peaks at 2923 cm^{-1} may indicate asymmetric stretching of an aliphatic ($-\text{CH}_2$) group or the

bending mode of the aromatic ($=CH$) group. Arrows in the graphical representation indicate these key absorption peaks (**Figure 7**).

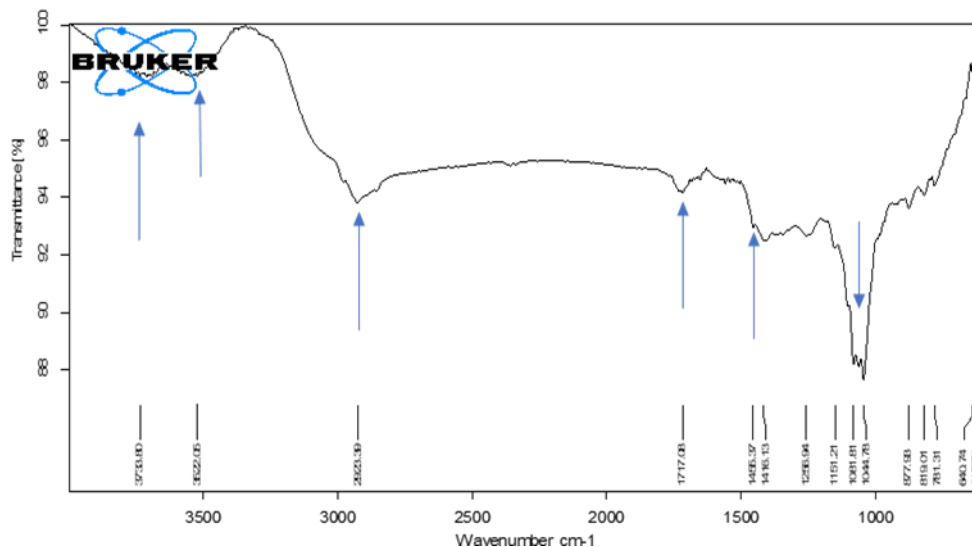


Figure 7. FTIR Spectroscopic analysis of jackfruit rag EE7

Analysis of Graphical representation of FTIR spectrum analysis of DE5.

The FTIR spectrum revealed significant peaks at 3734 cm^{-1} , 3278 cm^{-1} , 2981 cm^{-1} , 2380 cm^{-1} , 1653 cm^{-1} , 1083.88 cm^{-1} , and 1044 cm^{-1} . The peaks at 3734 cm^{-1} and 3278 cm^{-1} suggest the presence of alkaloids, indicated by the N–H stretch. The identified band at 2981 cm^{-1} may be due to the presence of alkynes, attributed to the $C\equiv C$ stretching vibrations. The C–H stretching vibration at 2938.72 cm^{-1} can be attributed to CH_2 and CH_3 groups, representing the presence of terpenes. The band at 1653 cm^{-1} could be linked to a deformed amino acid, flavonoids, aromatic ring, and stretching vibrations of $C=C$ groups. The peaks at 1083 cm^{-1} and 1044 cm^{-1} are likely due to C–O stretching vibrations, indicating the presence of an ester group or secondary alcohol (**Figure 8**).

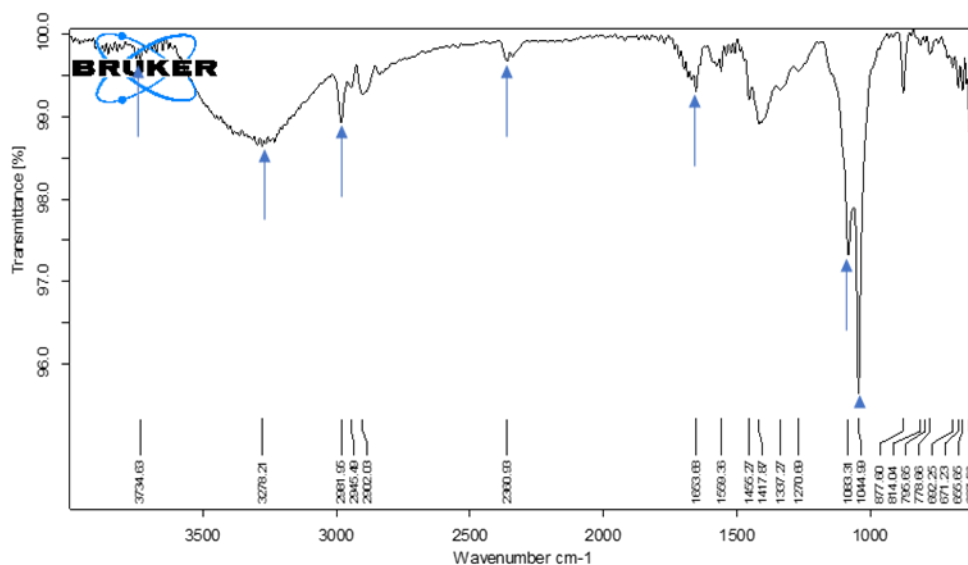


Figure 8. FTIR Spectroscopic analysis of jackfruit rag DE5

Identification of phenolic compounds using LC-MS analysis

UNIFI software identified polyphenols (**Table 3**) and their derivatives by comparing the data with a database of known compounds. Compounds belong to the group of phenolic compounds (3-Hydroxybenzylhydrazine), alkaloids (Tryptamine, Myoscorpine), benzoic acid esters (Butyl isobutyl phthalate, Dictamdiol), and three other compounds were putatively identified in this study. (**Figure 9 & 10**).

Table 3. Identification of compounds by LC-MS

SI No.	Component name	Observed neutral mass (Da)	Identification status	Natural mass (Da)	Observed mass m/z
1	3-Hydroxybenzylhydrazine	138.1	Identified	138.07931	183.0767
2	Candidate	144.1	Non-	-	144.2613

			Identified		
3	Tryptamine	160.1	Identified	160.1000 5	161.1067
4	Candidate	203.1	Non- Identified	-	203.1968
5	Candidate	205.1	Non- Identified	-	205.0733
6	Butyl isobutyl phthalate, Dictamdiol	278.1	Identified	278.1154 2	278.1144
7	Saussureamine C, Desacetylepaserri n	362.1	Identified	362.1841 7	385.1750
8	Myoscorpine and 2, ,6,10,14,18- Pntamethylicosa- 2,6,10,14,1 8- pentaene	381.1, 342.32	Identified,	381.2151 4	381.2161 381.2916

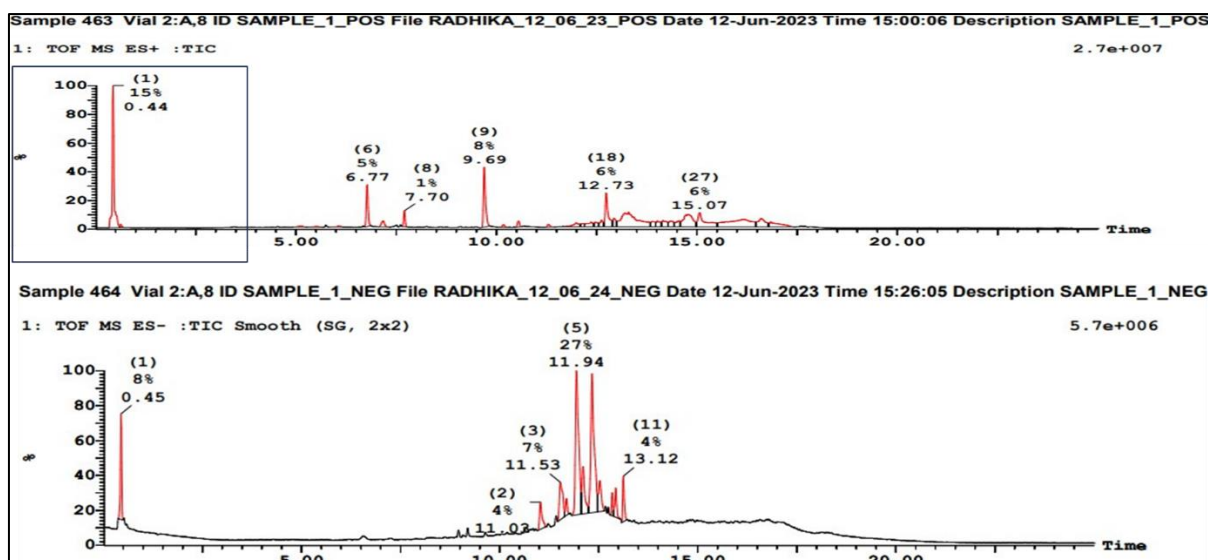


Figure 9. LC-MS chromatographic representation of DE5 in positive and negative mode.

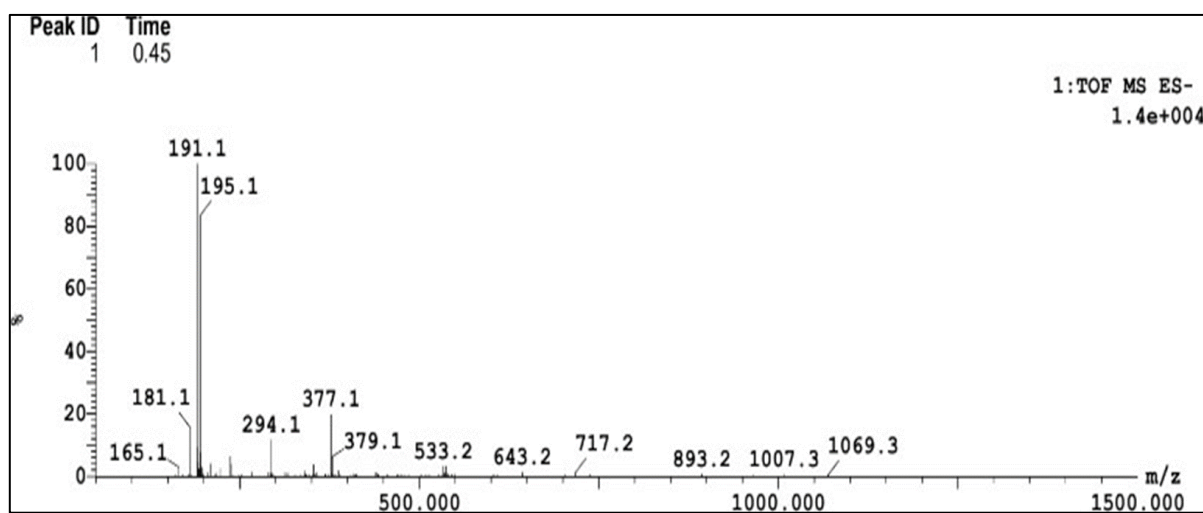


Figure 10. LC-MS chromatographic representation of DE5 in peak ID 1 of positive mode

Discussion

Jack tree fruit, scientifically known as *Artocarpus heterophyllus*, is a mulberry member of the Moraceae family. Apart from its nutritional significance, different parts of the jackfruit are known for their therapeutic characteristics [Jagtap and Bapat, (2010)].

Fruity arils have been associated with a variety of phytonutrients, including phenols, isoflavones, carotenoids, and saponins, which contribute to their immunomodulatory and antioxidant qualities (Chandrika et al., 2005; Biworo et al., 2015; Swami et al., 2012; Jagtap et al., 2010).

Previous studies have demonstrated that jackfruit leaf extracts exhibit antibacterial properties against *Salmonella typhimurium*, *Salmonella enterica*, *Bacillus cereus*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Escherichia coli*. The heartwood exhibits antibacterial properties against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Streptococcus pyogenes* while shell powder and extract from jackfruit seeds exhibit antibacterial properties against *L. monocytogenes* (Loizzo et al., 2010; Septama and Panichayupakaranant (2015); Khan et al., 2003; Sharma et al., 2015; Septama et al., 2017).

In traditional medicine, every part of the jackfruit tree is utilized for its therapeutic effects. They are utilized for their comforting properties and in treating inflammation, kidney stones, asthma, dermatitis, diarrhea, ulcers, infected wounds, anemia, fever, and malarial fever [de Araújo and de Paes (2012); Madaleno (2011)]. Because of their aphrodisiac qualities, the seeds are used to treat sexual issues [Fonseca (2016)].

In the present study, we aimed to investigate the potential antimicrobial properties of JFREs. Several studies have characterized various components of jackfruit, compiling extensive lists of their diverse applications. Previous studies in the literature have documented the antibacterial effectiveness of jackfruit tree against foodborne microorganisms, particularly crude methanolic (Khan et al., 2003) and

aqueous (Loizzo et al., 2010) extracts from leaves are efficient against *S. enterica*, *E. coli*, and *S. typhimurium*.

A total of 9 rag from jackfruits belonging to *A. heterophyllum* were collected which were subjected to a sequential extraction process based on the polarity of solvents. Solvents like petroleum ether, chloroform, ethanol, and water were used for the extraction. Among these methods, the petroleum ether (PE2) showed the highest yield (**Figure 2**).

The tested extracts PE1, CE1, EE5, EE7, and DE5 have demonstrated varying levels of inhibitory effects against specific bacterial strains. PE1 and CE1 showed inhibitory effects against *E. coli*, while EE5 displayed activity against *V. parahaemolyticus*, and DE5 showed activity against *Salmonella*. In this study, extracts derived from samples 1 and 5 showed antibacterial activity. Petroleum and chloroform extracts of sample 1 and aqueous extracts of sample 5 showed activity against food-borne pathogens such as *E. coli*, *Salmonella*, and *V. parahaemolyticus*. The MeOH extract in the Dhierllate (2021) investigation had the highest inhibitory concentration and bactericidal efficacy against the three strains of bacteria studied, at ≥ 7.2 mg/mL.

Furthermore, it was demonstrated that both the methanolic and ethanolic fractions were effective against methicillin-resistant *S. aureus* and other antibiotic-resistant bacteria (Karthi et al., 2009). These findings suggest that these extracts have the potential to be further explored and studied for their antimicrobial properties. Further research is necessary to fully comprehend their mechanisms of action, assess their safety, and explore potential applications in fighting bacterial infections. Dhierllate et al., 2021 highlight the bactericidal effect of jackfruit tree leaves at a concentration of 7.2 mg/mL on *E. coli* and *S. enterica* SE PT4 and the extracts' impact on antimicrobial activity.

To investigate the antibacterial activity of rag extract against *Shigella dysenteriae*, used a fly model to test the antibacterial activity of jackfruit rag extract against *S. dysenteriae* (Dhwani

et al., 2020). Their results indicated that the rag extract protected flies from enterotoxin-induced death by eliminating the bacteria in the gut. The antibacterial potential of dry leaf extracts against *S. enterica* and *E. coli*, as well as the interactions between extracts and fractions with conventional antimicrobials, were investigated by (Dhierllate et al., 2022). The findings demonstrated that the jackfruit extracts typically had an adverse effect on antimicrobials.

Fourier transform infrared transmission was incredibly helpful in identifying the presence of both inorganic and organic components in plants. Different therapeutic characteristics or biological activities can be detected by the existence of functional groups (Oliveira et al., 2016).

FTIR spectroscopy, utilizing the Fourier transform, investigates the vibrational characteristics of amino acids and cofactors, detecting subtle structural alterations. This technique's versatility enables direct examination of the vibrational attributes of various cofactors, amino acid side chains, and water molecules. Additionally, reaction-induced FTIR difference spectroscopy allows for the isolation of vibrations associated with individual chemical groups participating in specific reactions.

The FTIR analysis was conducted on the extracted samples that exhibited minimum inhibitory concentration (MIC) against specific bacterial strains. The FTIR spectra provided information about each extract's major functional groups and phytochemical compounds. The analysis revealed that the PE1, CE1, EE5, EE7 extract contained hydroxyl groups, possibly alkaloids, Carbonyl groups suggesting fatty acid or pectin residue, Peaks indicating C=C stretch and -CH₃/C-O groups related to flavonoids and hydrocyanides. Presence of aliphatic and aromatic groups. CE1 and EE7 also contained aliphatic and aromatic groups, and DE5 contained terpenes and secondary alcohols. Therefore, the FTIR analysis provided insights into the chemical composition of the jackfruit rag extracts, highlighting the presence of specific functional groups and phytochemical compounds. These findings contribute to the understanding of the extracts' potential antimicrobial properties. The present study utilized LCMS to identify compounds in the DE5 sample. The LC-MS analysis

of jackfruit rag extract identified a group of phenolic compounds (including 3-Hydroxybenzylhydrazine), alkaloids (such as Tryptamine and Myoscorpine), benzoic acid esters (including Butyl isobutyl phthalate and Dictamdiol), along with three additional compounds. These specific bioactive compounds might be responsible for the extract's antibacterial and other beneficial properties. LC-MS analysis of jackfruit rag extract has identified several polyphenols, while the chemical composition of jackfruit leaf and fruit extracts using this technique requires further investigation. Future work should explore other bioactivities like anti-inflammatory, anti-diabetic, and anti-cancer properties using in vitro and in vivo models. This could expand the potential applications of the extract in medicine and functional foods.

Conclusion

This novel work shows the quality of the extracts obtained by different extraction methods. The study demonstrated that the jackfruit rag could have a high content of phenolics, which have been reported to exhibit antimicrobial activities on bacteria of importance in agriculture. These findings will be valuable in identifying the diverse components of jackfruit. Extensive lists have documented its myriad uses and aim to investigate the potential antimicrobial properties of Jackfruit Residue Extracts (JFREs). This research delved into alternative reservoirs of antimicrobial compounds, primarily from discarded plant materials, offering cost-effective and feasible solutions to contemporary antimicrobial therapy challenges. The findings of this study will be valuable in identifying the diverse components of jackfruit. Extensive lists have documented its myriad uses and aim to investigate the potential antimicrobial properties of Jackfruit Residue Extracts (JFREs). This research delved into alternative reservoirs of antimicrobial compounds, primarily from discarded plant materials, offering cost-effective and feasible solutions to contemporary antimicrobial therapy challenges. To realize the commercial potential of jackfruit rag extract, research is needed to scale up production, ensure quality control, and assess

economic feasibility. Further studies are warranted to explore the extract's mechanisms of action, safety profile, and potential therapeutic uses.

Authorship statement

Akhila Dharnappa Sannejal: Conceptualization, Writing- Reviewing and Editing, **Radhika Suresh Jakkappagol:** Methodology, Writing- Original draft preparation. **Kavitha Guladahalli** **Manjunatha:** Methodology, Writing- Original draft preparation. **Abhirami Somasekharan:** Writing- Original draft preparation. **Ramith Ramu:** Writing-Reviewing and Editing, **Rajeshwari Vittal:** Supervision, Writing- Reviewing and Editing.

Data availability

The study data can be provided by the corresponding author upon reasonable request.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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