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## Extraction, Spectral Analysis and Pharmacological Action of Jackfruit Seeds Extract

# Vaibhav P. Uplanchiwar<sup>1</sup>, Trinayan Deka<sup>2</sup>, Ranjeet Yadav<sup>3</sup>, Sanjay Mishra<sup>4</sup>, B. Sanjeeb Kumar Patro<sup>5</sup>, Tahira Sultan<sup>6</sup>, Sohan Lal<sup>7</sup>, Shilpa Rani\*<sup>8</sup>

<sup>1</sup>Professor, Nagpur College of Pharmacy, Wanadongri, Hingna Road, Nagpur, Maharashtra, Pin Code: 441110

<sup>2</sup>Assistant Professor, Faculty of Pharmaceutical Science, Assam down town University, Panikhaiti, Guwahati, Assam, Pin Code: 781026

<sup>3</sup>Guest lecturer, Department of Botany, Naveen Govt College Jarhagaon Dist Mungeli, Pin Code: 495330

<sup>4</sup>Professor, Department of Biotechnology, SR Institute of Management & Technology, Bakshi ka Talab, NH 24, Sitapur Road, Lucknow, Pin Code: 226201

<sup>5</sup>Professor, College of Pharmaceutical Sciences, Mohuda, Berhampur, Dist: Ganjam, Odisha, Pin Code: 760002

<sup>6</sup>Assistant Professor, School of Pharmaceutical sciences, IFTM University, Moradabad, Pin Code: 244102

<sup>7</sup>Guest Lecture, School of studies in Botany, Shaheed Mahendra Karma Vishwavidyalaya Bastar, Jagdalpur, Chhattisgarh, Pin Code: 494001

<sup>8</sup>Assistant Professor, RIMT University, Delhi-Jalandhar GT Road (NH1, RIMT University Rd, Side, Sirhind, Mandi Gobindgarh, Punjab, Pin Code: 147301

## \*Corresponding Author:

Shilpa Rani

Designation and Affiliation: Assistant Professor, RIMT University, Delhi-Jalandhar GT Road (NH1, RIMT University Rd, Side, Sirhind, Mandi Gobindgarh, Punjab, Pin Code: 147301

Email Id: Shilpa35069@gmail.com

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#### **ABSTRACT**

Medicines made from plants have greatly benefited humanity by helping to treat bacterial diseases. In order to ensure safety and efficacy in the face of synthetic antibiotics' multidrug resistance, plants are now utilised in the pharmaceutical industry to manufacture innovative drugs. The current work sought to determine the antibacterial and phytochemical screening capability of A. heterophyllus ethanolic extracts. The produced ethanolic extract (EE) underwent phytochemical analysis, which revealed the presence of terpenoids, amino acids, alkaloids, glycosides, phenolic, and flavonoids. The DPPH and ABTS techniques were used to determine the antioxidant content and antioxidant studies of the ethanolic extract (EAE) of the root bark of A. heterophyllus Lam. By calculating the minimum inhibitory concentration (MIC) and zone of inhibition, the extract with the highest antioxidant property (EAE) was chosen for antibacterial investigations against bacterial strains. The findings of the research on antibacterial and antioxidant properties were encouraging.

Keywords: Pathogenic bacteria, A. heterophyllus Lam., Root bark, Antioxidant

## 1. INTRODUCTION

Because antibiotics are used indiscriminately to treat bacterial illnesses, pathogenic microorganisms frequently exhibit resistance to them [1]. Additionally, a variety of problems, including low efficacy, poor solubility, and a number of additional side effects, limit their use [2]. However, since ancient times, scientists have been interested in using plants as an alternative source of commercially available antibiotics because they are an endless source of numerous medications. The active components of herbal medications are a variety of potent secondary metabolites that are produced by plants [3, 4]. The antibacterial activity of plant extracts has been extensively documented in the literature [5, 6, 7]. Due to their multiple uses in diabetes, fungal infections, vector-borne diseases, and numerous other serious illnesses, plant-derived compounds have been utilised for decades [8–12]..

The World Health Organisation (WHO) [13] states that the best way to treat illnesses and give primary healthcare to roughly 75% of people in underdeveloped nations is through herbal medicines made from medicinal plants. Almost 78% of the novel medications that the FDA approved between 1983 and 1994 were herbal and semi-synthetically made from botanical sources [14]. Consequently, a number of microbially based disorders may be treated with some herbs that have been tested for antibacterial activity [15, 16].

Known as "Kathal," the jackfruit (Artocarpus species), which is a member of the Moraceae family, is a staple of the Indian cuisine. Jackfruit is accessible from spring until summer in the Indian market. Jackfruit pulp has a high nutritional content and is consumed fresh or added to fruit salads. The plant A. heterophyllus's root bark was chosen for our investigation, and its flavonoids and total phenolics were assessed [17]. The purpose of this study was to determine the plant's root bark extracts' antibacterial and antioxidant properties, as this had never been done before

#### 2. MATERIALS AND METHODS

**Collection of Plants:** Fresh roots of A. heterophyllus Lam, used for the study were collected from the Meerut district, Uttar Pradesh, India, during January 2024. The sample drug has been identified and authenticated by the botanist, Department of Botany.

**Extraction:** The roots of A. heterophyllus Lam were shade-dried. After the roots had dried, the bark was removed and ground into a powder. In a round-bottom flask, the powder was soaked in 95% ethanol for the entire night. After three hours of reflux, the solution was decanted off. Three extractions were made, or until a colourless extract was produced. A semisolid consistency was achieved by combining and condensing all of the extracts. The complete ethanolic extract was thus produced. Since the ethanolic yield was 11%, more research was done on it. [18]

**Preliminary Phytochemical Evaluation:** To identify the different phytoconstituents, a qualitative chemical analysis was performed on the ethanolic extract. Standard protocols were used to evaluate the drug's phytochemical properties. [8]

Estimation of Total Phenolics: The [16] was used to calculate the total phenolics in the extracts. Test tubes were filled with 200  $\mu$ l of samples. 0.8 millilitres of 7.5% sodium carbonate and one millilitre of Folin-Ciocalteu reagent were added. After mixing the tubes, they were let to stand for half an hour. At 765 nm, absorption was detected. The standard gallic acid graph was used to calculate the total phenolic content, which was then represented as gallic acid equivalents (GAE) in microgrammes per gramme of extract. [19]

Estimation of Total Flavonoids: A modified colorimetric technique was used to determine the extracts' total flavonoid content [17]. 75  $\mu$ l of a 5% NaNO2 solution and 1 ml of distilled water were combined with 1.0 ml of extracts. 75  $\mu$ l of a 10% AlCl3.H2O solution was added after five minutes. 0.5 cc of 1M sodium hydroxide was added after 5 minutes. After thoroughly mixing the solution, it was left for 15 minutes. The UV-Visible spectrophotometer was used to measure the rise in absorbance at 510 nm. The standard quercetin calibration curve was used to determine the total flavonoid concentration. Microgrammes of quercetin equivalents (QE) per gramme of extract were used to express the results. [20]

#### **Antioxidant Assay:**

#### **DPPH Assay:**

**Preparation of Standard Solution:** Ascorbic acid served as the standard. A 100 mg standard flask was filled with 100 mg of ascorbic acid to make up the volume. To make up the volume (stock solution), pipette 1 ml from this into a 10 mL standard flask. To get concentrations of 100, 200, 400, 600, and 800  $\mu$ g/mL, respectively, we pipetted out 1, 2, 4, 6, and 8 mL into separate 10 mL standard flasks. [21]

**Preparation of Test Sample:** Ten milligrammes of extract from each sample were dissolved in ten millilitres of ethanol to create stock solutions with a concentration of one milligramme per millilitre. To make up the volume (stock solution), pipette 1 mL of the solution into a 10 mL standard flask. To get concentrations of 100, 200, 400, 600, and 800  $\mu$ g/mL, respectively, solution pipetted 1, 2, 4, 6, and 8 mL from this stock into separate 10 mL standard flasks and filled the remaining volume with ethanol. [22]

**Preparation of DPPH Solution:** After 30 minutes, 2 mL of extracts and standard ascorbic acid at varying concentrations were mixed with 2 mL of ethanolic DPPH solution; the absorbance was measured at 517 nm. Using the formula, radical scavenging activity was determined.

 $Percentage\ inhibition = (absorbance\ of\ blank - sample/absorbance\ of\ blank) \times 100$ 

Every sample was examined three times before being averaged. A graph showing the percentage of inhibition vs concentration was used to determine the extract concentration needed to scavenge 50% of the radicles. [23]

#### **ABTS Assay:**

The ABTS radical cation preparation: In 50 millilitres of distilled water, ABTS 2 mM (0.0548g) was made. In distilled water, 70 mM potassium per sulphate (0.0189 g in 1 mL) was made. After two hours, 50 mL of ABTS and 200 μL of

potassium per sulphate were combined and utilised. This solution, known as the ABTS radical cation, was employed in the test. 0.3 mL of ABTS radical cation and 1.7 mL of phosphate buffer with a pH of 7.4 were added to 0.5 mL of extract at different doses. Ethanol was used as a control instead of extract. At 734 nm, the absorbance was measured. [24] Three duplicates of the experiment were conducted. The following formula was used to get the percentage inhibition:

% Inhibition = (Avg. OD of control - Avg. OD of Test)/ Avg. OD of control  $\times$  100

## **Determination of Antibacterial Assay**

**Microorganisms tested:** Three clinically significant human pathogenic bacterial strains—Bacillus subtilis (MTCC 4461), Escherichia coli (MTCC 7369), and Pseudomonas aeruginosa (MTCC 2464)—were included in the studied microorganisms. Every bacterial strain was kept on nutrient agar slants at 4 °C and cultivated in nutrient broth (Hi-Media, M002) at 37 °C. [25]

**Inoculum standardization:** For roughly 16 hours, all bacterial strains were inoculated in Müeller-Hinton broth (pH 7.4). Using a spectrophotometer, the concentration of the suspensions was brought to 0.5 (optical density).

Assay of antibacterial activity using agar well diffusion method: The Agar well diffusion method was used to assess the crude and solvent extracts' antimicrobial activity [26]. Sterile petri dishes were filled with 20 millilitres of nutrient agar that had been sterilised. Following solidification, sterile spreaders were used to inoculate 100  $\mu$ l of each isolate's standardised inoculate onto nutrient agar plates. A sterile gel puncher with a 6 mm diameter was used to punch the wells over the agar plates. Each extract was added to a different well with 100  $\mu$ l. The extracts were dissolved in 1% (v/v) dimethylsulfoxide (DMSO), which served as the solvent extract's negative control. Aqueous and ethanolic extracts at four different concentrations (50, 100, 200, and 400  $\mu$ g/ml) were evaluated. For 24 hours, plates were incubated at 37 °C. [27] To guarantee dependability, triplets of the experiment were kept for every bacterial strain. Following incubation, the diameter of the circular inhibitory zones that developed around each well was measured and reported in millimetres.

Antibiotic susceptibility test: Using standard antimicrobial sensitivity testing drugs acquired from Hi-Media Laboratories Limited, Bombay, an antibiogram was conducted using the disc diffusion method. A sterile swab spreader was used to wipe the test organisms across the surface of the hardened agar plates after the plates had been prepared. After that, the inoculated agar plates were impregnated with standard antibiotic discs, and they were incubated for 24 hours at 37 °C. The zone of inhibition that the test bacteria created around the conventional antibiotic discs was then measured [28].

Minimum inhibitory concentration (MIC): Plant extracts cannot be precisely tested using standard antimicrobial susceptibility test procedures that have been authorised by the National Committee for Clinical Laboratory Science (NCCLS) [29] for evaluating conventional medications. As a result, adjustments have been made to assess the antibacterial activity of plant-based active principles. With minor adjustments, the broth dilution method was used to estimate the minimum inhibitory concentration (MIC) for each extract exhibiting antibacterial activity against the test isolates [30, 31]. Müeller-Hinton broth was used to dilute each culture. To achieve turbidity of 0.5 McFarland standards, the concentration of test cultures was adjusted. The test tubes were filled with nourishing broth and an equal volume (0.5 ml) of each extract (by serial dilutions from the stock solution of ethanol extract). Each test tube included three extracts with final concentrations between 40, 35, 30, 25, 20, 10, 5, 2.5, 1.25, and 0.625 µg/ml. In particular, each tube received 0.1 ml of standardised inoculum (5 × 105 CFU/ml). Every tube was incubated at 37 °C for 24 hours. Following incubation, statistics were recorded and growth was analysed. The lowest extract concentration at which there was no growth in the tube was used to calculate the MIC values. Experiment was repeated thrice for authentication of the data. [32]

## 3. RESULTS AND DISCUSSION

**Spectrophotometric Analysis:** The detection of absorption peaks between 200 and 400 nm suggests the presence of a conjugate double bond and a C=O group, indicating that the molecule is most likely saturated. >C=O, >C=C-O-, or >C=C-N<, among other things, may be present if there is a faint peak (=10~100) between 270 and 350 nm and no additional peaks are found over 200 nm. The n-\* transition was the cause of the weak peak.

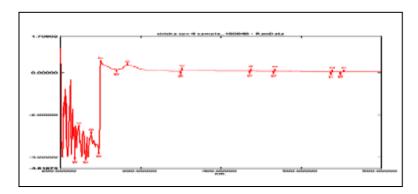


Fig. 1: Uv-Vis Absorption Spectra's of A. heterophyllus extract

**Preliminary phytochemical:** Alkaloids, glycosides, phenolic, flavonoids, carbohydrates, proteins, amino acids, terpenoids, sterols, and saponins were among the phyto-constituents identified by qualitative chemical analysis of the ethanolic extract's preliminary phytochemical evaluation.

Quantitative analysis: The extract's quantitative analysis revealed improvement as concentrations increased from 25 mg/ml to 100 mg/ml. At 100 mg/ml, the phenolic content of the jackfruit extract was found to be  $0.41 \pm 0.02$  µg gallic acid equivalents g-1 for A. heterophyllus. Phenolic chemicals belong to a class of antioxidant agents that both serve as terminators of free radicals and slow down the oxidative destruction of lipids. Additionally, phenolic content and antioxidant activity in specific fruits and vegetables have been found to be strongly correlated. Thus, Artocarpus ethanolic extract's nutritional and health potential was enhanced by the presence of phenolic components. In A. heterophyllus, the screening flavonoid concentration of 100 mg/ml of Artocarpus ethanolic extract was  $4.43 \pm 0.01$  µg quercetin equivalents g-1. Additionally, the presence of flavonoids, which are phenolic chemicals, in Artocarpus significantly improves its potential for both health and the economy. This is consistent with earlier research suggesting flavonoids have an antioxidant effect through a scavenging or chelating process and are thought to help against heart disease and cancer.

Antioxidant Assay: The percentage inhibition achieved with the standard, as shown in Table 1, was compared to the percentage inhibition obtained with the various amounts of sample extracts in the DPPH experiment. The extract demonstrated a dose-dependent pattern in DPPH radical scavenging, as evidenced by a decrease in purple colour development, when the DPPH+ scavenging properties of CE and EAE were examined in the concentration range of 100-800 ( $\mu$ g/mL) and standard. The extract's IC50 values were determined to be CE = 112.5  $\mu$ g/mL and EAE = 98.78  $\mu$ g/mL. Ascorbic acid's IC50 value was determined to be 66.28  $\mu$ g/mL. The ethanolic extract of A. heterophyllus's DPPH+ radical scavenging capabilities. In comparison to ascorbic acid (66.28( $\mu$ g/mL)), EAE (98.78( $\mu$ g/mL)) demonstrated a favourable outcome. The ethanolic extract exhibited good antioxidant capability, according to the estimation of antioxidant activity using the DPPH radical scavenging method.

Table 1: % Inhibition By Standard Ascorbic Acid And Of CE And EAE of A. Heterophyllus Lam. For DPPH Assay

Sample	Concentration (μg/mL)	Absorbance*	% Inhibition	IC <sub>50</sub> (μg/mL)	
Standard (ascorbic acid)	100	$0.309 \pm 0.001$	15.78	66.28	
	200	$0.218 \pm 0.003$	40.11		
	400	$0.189 \pm 0.002$	47.23		
	600	$0.165 \pm 0.002$	55.37		
	800	$0.092 \pm 0.001$	76.87		
СЕ	100	$1.754 \pm 0.02$	1.03	112.5	
	200	$1.432 \pm 0.01$	14.22		
	400	$1.268 \pm 0.02$	25.23		
	600	$0.849 \pm 0.01$	49.23		
	800	$0.806 \pm 0.03$	56.86		
EAE	100	$0.229 \pm 0.0002$	34.75	98.78	
	200	$0.116 \pm 0.0001$	67.54		
	400	$0.051 \pm 0.0001$	82.17		
	600	$0.043 \pm 0.0001$	86.34		
	800	$0.036 \pm 0.0002$	90.06		

Table 2 lists the percentage inhibition obtained in the ABTS antioxidant assay for various sample extract concentrations in

comparison to the percentage inhibition obtained using the standard. A protonated radical called ABTS+ has a distinctive absorption maximum around 734 nm, which reduces proton radical scavenging. The activity of the examined extracts was expressed in the ABTS+ cation radical scavenging method as a micromolar equivalent of an ascorbic acid solution with an antioxidant equivalent to 1g dry matter of the experimentally investigated sample.

Table 2: % Inhibition by Standard Ascorbic Acid and Of CE and EAE of A. Heterophyllus Lam. for ABTS Assay

Sample	Concentration (μg/mL)	Absorbance*	% Inhibition	IC <sub>50</sub> (μg/mL)	
Standard (ascorbic acid)	125	$0.095 \pm 0.0003$	7.43	967.23	
	250	$0.078 \pm 0.0004$	21.07		
	500	$0.056 \pm 0.0021$	42.56		
	1000	$0.035 \pm 0.0009$	63.54		
	2000	$0.014 \pm 0.0004$	81.64		
CE	125	$0.081 \pm 0.0005$	15.54	913.55	
	250	$0.058 \pm 0.0003$	38.87		
	500	$0.052 \pm 0.0005$	43.52		
	1000	$0.029 \pm 0.0002$	65.53		
	2000	$0.026 \pm 0.0003$	68.76		
EAE	125	$0.052 \pm 0.0002$	41.43	265.75	
	250	$0.046 \pm 0.0004$	48.64		
	500	$0.035 \pm 0.0002$	49.87		
	1000	$0.031 \pm 0.0002$	67.11		
	2000	$0.015 \pm 0.0003$	83.23		

#### **Antibacterial Activity**

The three harmful microorganisms that were investigated were significantly inhibited by jackfruit extracts. All of the bacterial isolates in this experiment were inhibited by the ethanolic extract (400  $\mu$ g/ml), but P. aeruginosa was the most sensitive, with an inhibition zone of 29.33  $\pm$  0.33 mm. B. subtilis and E. coli (MTCC 739) followed, with inhibition zones of 27.00  $\pm$  0.00 mm and 23.67  $\pm$  0.33 mm in diameter, respectively. Conversely, none of the examined bacteria's growth was inhibited by negative controls, such as pure water and DMSO (Table 3). All of the bacterial isolates in this study were resistant to common antibiotics such as Ampicillin (10  $\mu$ g/disc) and Penicillin G (10  $\mu$ g/disc), according to the antibiogram results. All gramnegative bacteria were susceptible to Gentamycin (30  $\mu$ g/disc), and both gram-positive and gram-negative bacteria were more susceptible to each of the tested antibiotics. Additionally, the bacterial strains displayed varying antibiotic susceptibilities (Table 3). Gentamycin (30  $\mu$ g/disc) suppressed the growth of B. subtilis with the highest inhibition zone, while it was most effective against E. coli. Additionally, the results showed that the extracts' antibacterial effectiveness rose as the concentration employed increased. Serial dilution of the leaf extracts was used to assess MIC, and the findings showed that very low quantities were effective against the tested bacterial strains (Table 3).

Table 3: Antibacterial Bioassay of different extracts of leaves of Combretum album and susceptibility test of some standard antibiotics

Bacterial	Diameter of inhibition zone(mm)			Antibiotics a			
strains	Strains Ethanol extract (μg/ml)						
	100 μl/well				Ampicillin	Penicillin G	Gentamycin
	50	100	200	400	(10 μg disc)	(10 µg/disc)	(30 µg/disc)

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B. subtilis	$23.67 \pm 0.54$	$24.33 \pm 0.29$	$24.61 \pm 0.54$	$27.00 \pm 0.00$	$0.00\pm0.00$	$24.7 \pm 0.88$	$0.00\pm0.01$
E. coli	$17.33 \pm 0.43$	$20.09 \pm 0.56$	$21.32 \pm 0.23$	$23.67 \pm 0.32$	$19.67 \pm 0.87$	$23.67 \pm 0.88$	$31.76 \pm 0.01$
P. aeruginosa	$20.00 \pm 0.54$	$21.65 \pm 0.78$	$25.16 \pm 0.65$	$29.33 \pm 0.45$	$17.87 \pm 1.45$	$27.33 \pm 0.67$	$27.98 \pm 0.03$

a Antibiotics were used based on their spectrum of activity i.e., their efficacy against gram positive, gram negative or both

#### 4. CONCLUSION

For the investigation, dried root bark from A. heterophyllus Lam was chosen. The dried root bark of A. heterophyllus Lam was extracted using ethanol in the current investigation. When compared to the standard ascorbic acid, antioxidant activity experiments utilising the DPPH and ABTS radical scavenging methods revealed that the ethanolic extract had excellent antioxidant activity and the chloroform fraction had good antioxidant activity. A significant problem in the treatment of bacterial infections worldwide is the rise in resistance to commercially available antibiotics. The root bark of A. heterophyllus Lam may be a possible source for herbal medication compositions that combat harmful microorganisms, according to the findings of the aforementioned experiment. In order to create a novel therapeutic agent to combat infectious diseases, phytochemical group-wise screening and the separation of bioactive chemicals will be further studied in the future.

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