

**ANALYSIS OF ANTIOXIDANT AND ANTI-MICROBIAL ACTIVITY IN
METHANOLIC EXTRACT OF *ARTOCARPUS HETEROPHYLLUS* SEED AND
FRUIT**

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ABSTRACT

This study looks into the antibacterial and antioxidant properties of methanolic extracts made from *Artocarpus heterophyllus* (jackfruit) seeds and fruits. By scavenging radicals with DPPH (2,2-diphenyl-1-picrylhydrazyl), the antioxidant activity was assessed, while the antimicrobial activity was assessed against a panel of bacterial and fungal strains using the agar well diffusion method. Results revealed significant antioxidant activity in both seed and fruit extracts, with higher scavenging potential observed in the seed extract. Additionally, the methanolic extracts exhibited promising antimicrobial activity against various bacterial and fungal strains, suggesting their potential as natural antimicrobial agents. Further investigation into the bioactive components responsible for these activities is warranted for potential pharmaceutical and food applications.

Keywords: *Artocarpus heterophyllus*, Phytochemicals, Radical scavenging assay, Antimicrobial, Bioactive components.

1. INTRODUCTION

In several Asian nations, the jackfruit, also known as *Artocarpus heterophyllus* is a popular fruit. Although people don't use or recognize jackfruit seeds as often, they have significant nutritional value and may be used as a functional food ingredient. To the greatest extent of the authors' knowledge, a significant amount of research has been done on the makeup of jackfruit seeds and their potential health effects [1]. Since synthetic antibiotics have proven unsuccessful against a number of pathogenic species due to rising drug resistance, antibacterial substances made from the plant have drawn more interest recently. Over 900 types of valuable medical plants are rumored to be discovered in Nepal out of the nearly seven thousand types of plants with medicinal uses identified worldwide [2]. Apart from its antimicrobial properties, *A. heterophyllus* has been found to have anti-inflammatory, antioxidant, anticholinergic, anti-diabetic, immune modulatory, protease inhibition, estrogen regulation, and melanin biosynthesis inhibition properties, according to various pharmacological research investigations of plant parts [3]. Despite being a plant that produces latex, jackfruit's latex is not very sought after by manufacturers. It comes out of the leaves, bark, roots, and fruit of the jack tree as a milky white liquid that's quite sticky to the touch [4]. Within 15 minutes of eating dry jackfruit, a 34-year-old woman had dyspnea, facial angioedema, coughing, urticaria, and chest pain. This encourages the myth among a lot that jackfruit latex only poses health risks to people

and has no positive health benefits. Still, as Swami et al. reviewed [5]. Snakebites, pharyngitis, ocular infections, and even hormonal swelling can all be relieved with jackfruit latex. Furthermore, the use of a vinegar and jackfruit latex mixture can help prevent the worsening of wound abscesses [6]. It is also reported that 40–50% of medications contain synthetic or direct replicas of plant components. Poorer communities can combat illness more affordably by using therapeutic plants. It is well known that the majority of developing nations utilize traditional medicine and medicinal plants as a normative foundation for maintaining good health [7].

The plant *Artocarpus heterophyllus*, a native of India and Malaysia, was introduced by Arabs to Africa, then to South America, where it eventually became acclimated in Mexico. In Southeast Asia, it has significant commercial, nutritional, and therapeutic benefits. In traditional medicine, it was used to treat cough, dermatitis, ulcers, wound healing, and asthma [8]. The statements above provide compelling evidence that plants carrying latex, like jackfruit, are still considered to have magical or medicinal value by native American tribes, who feel that properly prepared plant latex can heal a wide range of illnesses. Many people are unaware of the precise chemical makeup and functional identity of jackfruit latex, which unintentionally blurs the boundaries between what is now known and what could be used for the benefit of humans. In order to determine the use of latex from jackfruit in the scientific and medical fields, the current study will proceed to look into the bioactivity of the material [9].

2. MATERIALS AND METHODS

2.1 Collection of plant material

Freshly picked *Artocarpus heterophyllus* fruit was gathered from Tamil Nadu's Tirupattur area. After being cleaned with distilled water, the seeds and fruit of *Artocarpus heterophyllus* were allowed to air dry under shade. Using a blender, the fruit and seed are ground into fine powders separately. After that, the fine powders is employed for other experiments and kept in a closed bottle.

2.2 Extraction of plant sample

Using a Soxhlet apparatus, powdered sample of *Artocarpus heterophyllus* fruit and seed were extracted using 100 milliliters of methanol. A rotatory evaporator was used to concentrate the solvent, and the extract was kept for later use at 4°C.

2.3 Chemicals

Methanol, DPPH, Ascorbic acid, Nutrient broth, Muller Hinton agar, DMSO.

2.4 Qualitative Phytochemical analysis

The plant extract was tested for detecting various Phytochemicals present in the methanolic extract *Artocarpus heterophyllus* using the standard method [10] [11].

1) Test for carbohydrates

A) Molish's test

Plant extract was treated with few drops of Alpha naphthol and concentrated sulphuric acid added to the mixture in a slanting position. Observance of violet colour ring indicates the presence of carbohydrates.

B) Benedict's test

0.5 ml of extract was treated with 0.5 ml of Benedict's reagent and kept for water bath for 2 minutes. Formation of precipitates indicates the presence of carbohydrates.

2) Test for tannins**A) Ferric chloride test**

A tiny amount of extract was warmed in a bath of water after being combined with water. After the mixture was filtered, the filtrate was mixed with 5% ferric chloride. It formed a dark green color. It suggests that tannins are present.

B) Lead acetate test

The sample solution was mixed with 3 milliliters of 10% lead acetate. Tannic acid is indicated by the formation of a large, white precipitate.

3) Test for saponins

2 ml of distilled water was added with the sample solution and shakes well. Formation of foams indicates the presence of saponins.

4) Test for alkaloids**A) Mayer's test**

The sample solution is treated with 2 drops of Mayer's reagent. Formation of white creamy precipitate indicates the presence of alkaloids.

B) Wagner's test

Few drops of Wagner's reagent was added with the sample. Formation of reddish brown precipitate indicates the presence of alkaloids.

5) Test for Flavonoids

The sample solution is treated with 1 ml of 2N sodium hydroxide. Formation of yellow colour indicates the presence of flavonoids.

6) Test for glycosides

The sample solution is treated with 3 ml of chloroform and 10% ammonia. Formation of pink colour indicates the presence of glycosides.

7) Test for quinones

1 ml of concentrated sulphuric acid is added to the sample in slanting position. Formation of red colour indicates the presence of quinones.

8) Test for Phenols

10% ferric chloride is added to a small amount of the sample solution. Phenols are present when a blue or green color begins to form.

9) Test for terpenoids

The sample solution is added with 2 ml of chloroform and treated with concentrated Sulphuric acid. Formation of red brown colour indicates the presence of terpenoids.

10) Test for steroids

Few drops of concentrated sulphuric acid is added to the sample solution in a slanting position. Occurrence of brown ring indicates the presence of steroids.

2.5 ANTIOXIDANT ACTIVITY

The free radical-scavenging capacity of 1,1-diphenyl-2-picrylhydrazyl, or DPPH, is used to measure antioxidant activity. DPPH was used to assess the methanolic extract of *Artocarpus heterophyllus* seed and fruit its capacity to scavenging free radicals. In 95% methanol, the DPPH solution (0.006% w/v) was made. The methanolic extract of *Artocarpus heterophyllus* seed and fruit was produced in methanol at varying doses (10, 20, 30, 40, and 50 µg/ml). In the dark, 1ml of DPPH solution and 3ml of various concentrations of methanolic extract were combined. To produce ascorbic acid, a potent antioxidant, methanolic acid is utilized as a

standard at concentrations of 10, 20, 30, 40, and 50 µg/ml. A standard ascorbic acid solution with a configurable concentration and 3 milliliters of methanolic extract were combined [12].

DPPH radical scavenging activity (%) = (Control OD-Sample OD)/Control OD x 100

2.6 ANTIBACTERIAL ACTIVITY

Assay of antibacterial activity of plant extracts,

Plant extracts were tested for antibacterial activity using the standard diffusion method based on agar wells. Different quantities (25, 50, 75, and 100 µg/ml) of methanolic extract were produced using 2% dimethyl sulphoxide (DMSO). Using the spread plate method, 10µl of 24-hour test cultures, including *Escherichia coli* and *Staphylococcus aureus*, were placed onto the appropriate Muller-Hinton agar medium. After that, the plates were incubated for a full day at 37°C. A zone of inhibition grew around the well, and its diameter was used to measure the antibacterial activity [13].

2.7 ANTIFUNGAL ACTIVITY

It was decided to assess antifungal activity using the conventional agar well diffusion method. Different amounts (25, 50, 75, and 100 µg/ml) of *Artocarpus heterophyllus* fruit and seed were generated using 2% DMSO (dimethyl sulphoxide). Test cultures, like those of *Candida albicans*, were cultivated for 72 hours on the proper sabouraud dextrose agar medium using the spread plate technique. The plates were subsequently incubated at room temperature for four to five days. The antifungal activity was quantified by measuring the diameter of the zone of inhibition that grew around the well [14].

3. RESULTS AND DISCUSSION

3.1 PHYTOCHEMICAL ANALYSIS OF *ARTOCARPUS HETEROPHYLLUS*

The methanolic extract of *Artocarpus heterophyllus* leaf was go through into the analysis of phytochemical compounds. The study has done to find out the distinct phytochemicals such as carbohydrates, tannins, flavonoids, saponin, steroids, terpenoids.

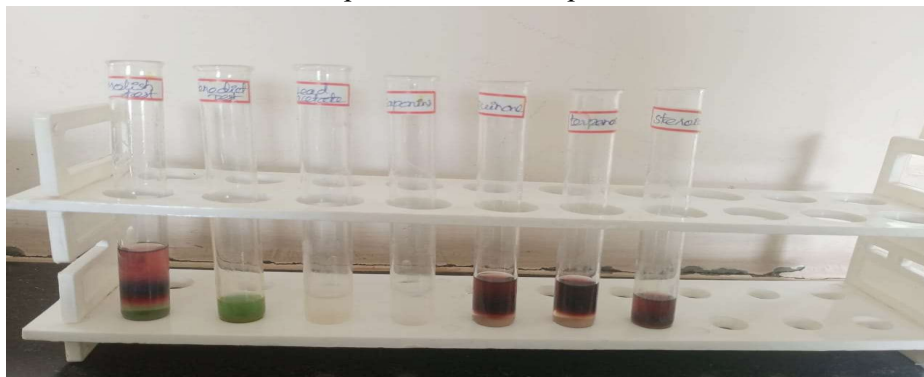


Figure 1. Qualitative phytochemical analysis of methylated seed extract



Figure.2 Methanolic fruit extract: a qualitative phytochemical analysis (FRUIT)

Table: 1 Phytochemical analysis of *Artocarpus heterophyllus* seed and fruit extracts

S. No	Phytochemical Compounds	<i>Artocarpus heterophyllus</i> SEED	<i>Artocarpus heterophyllus</i> FRUIT
1.	Carbohydrate	+	+
2.	Alkaloids	-	-
3.	Glycosides	-	-
4.	Phenols	-	-
5.	Flavonoids	-	+
6.	Saponins	+	+
7.	Steroids	+	+
8.	Tannins	+	+
9.	Terpenoids	+	+
10.	Quinones	+	+

Symbol (+) indicate present and (-) indicate absence

3.1 ANTIOXIDANT ACTIVITY

Figure 3 The DPPH scavenging radical and reducing ability identified in *the Artocarpus Heterophyllus* fruit and seed methanolic extract. A study regarding the antioxidant properties of the bioactive evidence has been carried out utilizing a stable DPPH approach. The sample's scavenging potential was determined through the measurement of its absorbance at 517 nanometers. To contrast the potential benefits of *Artocarpus heterophyllus* leaf extract, ascorbic acid was employed as a benchmark. Comparing the DPPH antioxidant activity of *Artocarpus heterophyllus* methanolic leaf extract to standard ascorbic acid, it was found that the extract was the most potent at 100µg/ml, with a scavenging potential of over 90%.

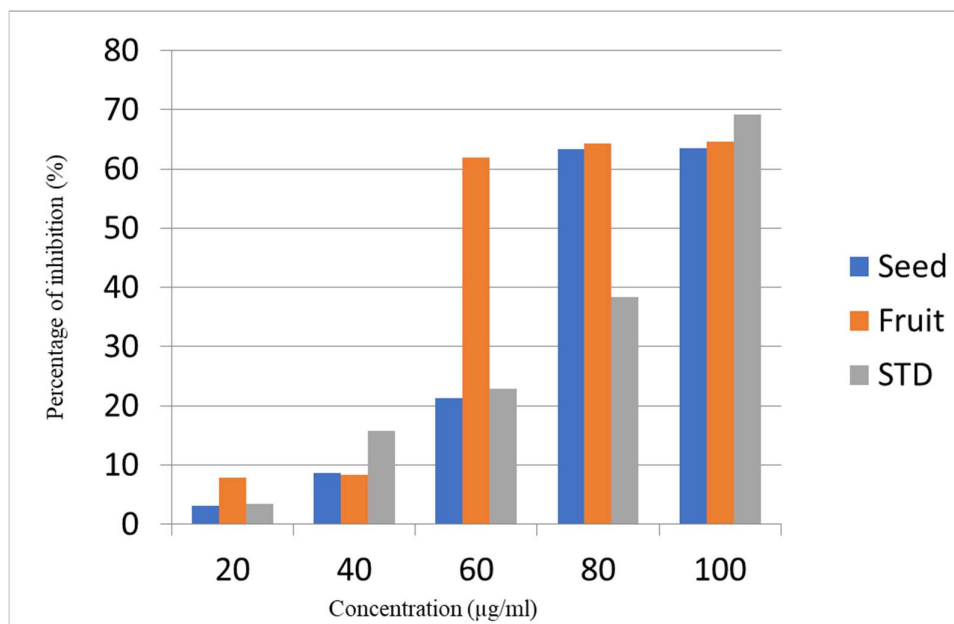


Figure. 3 Antioxidant activity in methanolic extract of *Artocarpus Heterophyllus*

3.3 ANTIBACTERIAL ACTIVITY

Figure 4 illustrates the antibacterial (gram positive and gram negative) activity of *Artocarpus heterophyllus* methanol extract. Zone of inhibition (mm) against gram-positive and gram-negative bacteria on *Artocarpus heterophyllus* fruit and seed methanolic extract is shown in Table 2.

Table 2: Antibacterial activity of methanolic extracts of *Artocarpus heterophyllus* seed and fruit

Name of the Microbes	Methanolic extract of <i>Artocarpus seed</i>				Methanolic extract of <i>Artocarpus fruit</i>			
	25	50	75	100	25	50	75	100
Concentration (µg/ml)								
Bacteria	Zone of Inhibition (mm)							
<i>S. aureus</i>	8	10	11	12	-	-	9	11
<i>E. coli</i>	-	-	-	-	-	-	-	-

The top most antibacterial activity of *Artocarpus heterophyllus* leaves, was found in 150 µg/ml of methanolic extract was screened. The greater inhibition zone was found in methanolic extract of *Artocarpus heterophyllus* s. aureus (12 mm). And there is no formation of zone of inhibition against gram negative bacteria (*E. coli*).

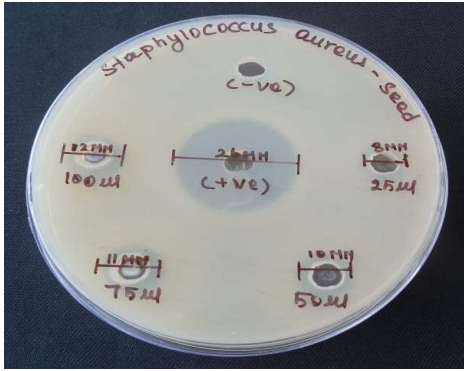


Figure 4 (a)



Figure 4 (b)

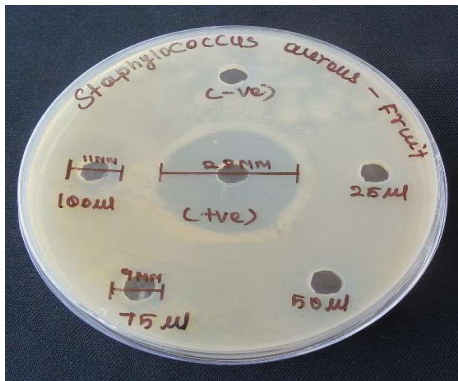


Figure 4 (c)

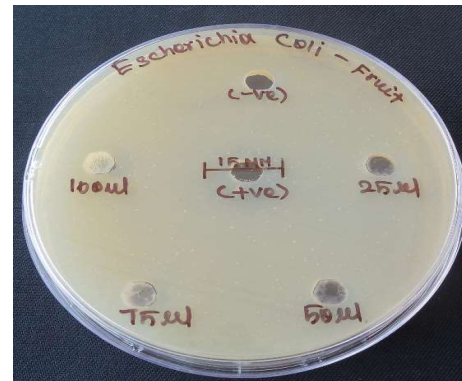


Figure 4 (d)

3.4 ANTIFUNGAL ACTIVITY

Antifungal activity of methanol extract of *Artocarpus heterophyllus* is sketched in figure 5.

Table 3: Zone of inhibition (mm) against Fungal on methanolic extract of leaf *Artocarpus heterophyllus*.

Table 3: Antifungal activity of methanolic extracts of *Artocarpus heterophyllus* seed and fruit

Name of the Microbes	Methanolic extract of <i>Artocarpus heterophyllus</i> seed				Methanolic extract of <i>Artocarpus heterophyllus</i> fruit			
	Concentration (µg/ml)	25	50	75	100	25	50	75
Fungi	Zone of Inhibition (mm)							

<i>Candida albicans</i>	-	-	-	10	-	-	10	13
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The antifungal activity of *Artocarpus heterophyllus* against *Candida albicans* shown maximum 13mm of inhibition zone formation. The methanolic extract of fruit has shown significant potential against *Candida albicans* than the methanolic extract of seed of *Artocarpus heterophyllus*.



Figure 5 (a)

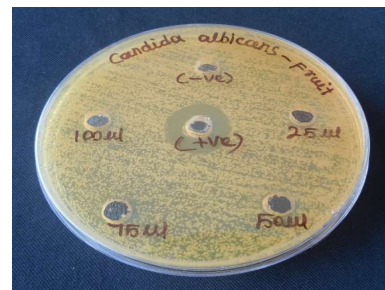


Figure 5 (b)

4. CONCLUSION

The Analysis of Initial Screening for Phytochemicals administering an extract of methanol of *Artocarpus heterophyllus* concludes that presence of Tannins, Flavonoids, Quinone, Terpenoid, Steriod. And methanolic extract of seed was reveals that Tannins, Saponins, Quinone, Terpenoids, Steroids. The methanolic extract's potency for antioxidants shows significant ranges in contrast with DPPH-by-DPPH free radical scavenging method. The methanolic extract of fruit has shown significant potential against *Candida albicans* than the methanolic extract of seed of *Artocarpus heterophyllus*. The current study revealed that anti bacterial and anti fungal activity against human microbes.

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REFERENCES

1. Prakash O, Kumar R, Mishra A, Gupta R (2009) *Artocarpus heterophyllus* (Jackfruit): *An overview. Pharmacogn Res* 3(6): 353-358
2. Akgul C, Saglikoglu G: Antibacterial activity of crude methanolic extract and its fractions of aerial parts of *Anthemis tinctoria*. *Ind J Biochem Biophy*. 2005, 42: 395-397.
3. Mandal V, Mohan Y, Hemalatha S: Microwave assisted extraction—an innovative and promising extraction tool for medicinal plant research. *Pharmacognosy Reviews*. 2007, 1(1):7-18.
4. Shrestha P et al: Phytochemical screening, antimicrobial activity and cytotoxicity of Nepalese medicinal plants *Swertia chirayita* and *Dendrobium amoenum*. *NJB* 3.1. 2015: 48-57.
5. Akowuah, G.A.; Ismail, Z.; Norhayati, I.; Sadikun, A. The effects of different extraction solvents of varying polarities of polyphenols of *Orthosiphon stamineus* and evaluation of the free radical-scavenging activity. *Food Chem*. 2005, 93, 311-317.

6. Haq, N. Fruits for the Future 10. Jackfruit (*Artocarpus heterophyllus*). Southampton Centre for Underutilised Crops: Southampton, England, 2006; p. 192.
7. Culture and Health, Orientation Texts – World Decade for Cultural Development 1988 – 1997, Document CLT/DEC/PRO – 1996, Paris, France, pg. 129.
8. Jagtap UB, Bapat VA (2010) Artocarpus: A review of traditional uses, phytochemistry and pharmacology. *J Ethnopharmacol* . 129(2):142-166.
9. Samrot, A.V.; Sahiti, K.; Bhavya, K.S.; Suvedhaa B. Synthesis of Plant Latex Based Hybrid Nanocarriers Using Surfactants for Curcumin Delivery. *J Clust Sci*. 2019. 30, 281–296.
10. Harborne JB (1998) phytochemical methods, London. rd3 Edn., Chapman and Hall, Ltd. pp. 1-302
11. Kokate CK, Purohit AP, Gokhale SB. Textbook of pharmacognosy, Nirali prakasan: Pune, 2002; pp. 1-4.
12. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakrin J Sci. Technol*. 2004; 26: 211-16.
13. NCCLS. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 2000; 5th ed. NCCLS document M7-A5. NCCLS, Wayne,
14. Pachlumbaum, A., Mauch, F., Vögeli, U. & Boller, T. (1986). *Nature (London)*, 324, 365–367.