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PHYTOCHEMICAL PROFILING AND ANTIBACTERIAL EFFICACY OF ETHYL ACETATE EXTRACT OF *CARICA PAPAYA* SEEDS (VIA LIQUID–LIQUID EXTRACTION): A KIRBY–BAUER METHOD INVESTIGATION

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ABSTRACT

Aim: This study aimed to investigate the phytochemical profile and evaluate the *in vitro* antibacterial activity of the ethyl acetate extract of *Carica papaya* seeds, obtained via liquid-liquid extraction (LLE), against selected Gram-positive and Gram-negative bacteria. **Methods:** Dried and powdered papaya seeds were subjected to LLE using ethyl acetate as the solvent. The extract was screened for major phytochemical constituents using standard qualitative tests. The antibacterial efficacy was assessed against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) using the Kirby-Bauer disc diffusion method. A concentration of 1 g/mL of the extract was tested, with Amoxicillin (10 µg/disc) as the positive control and ethyl acetate as the negative control. The zones of inhibition were measured after 24 hours of incubation at 37°C. **Results:** Phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, saponins, and carbohydrates. The ethyl acetate extract exhibited significant antibacterial activity against *S. aureus*, producing a mean inhibition zone of 15 mm, which was larger than that produced by the standard Amoxicillin (10 mm). In contrast, no zone of inhibition was observed against *E. coli* for both the extract and the standard drug under the tested conditions. **Conclusion:** The ethyl acetate extract of *Carica papaya* seeds demonstrates potent and selective antibacterial activity against Gram-positive *S. aureus*, outperforming Amoxicillin in this assay. This activity is likely due to its rich phytochemical composition. The lack of effect on *E. coli* highlights its spectrum-specific action. These findings validate the traditional use of papaya seeds and suggest their potential as a source for developing natural antibacterial agents against Gram-positive infections, warranting further investigation into active compound isolation and the mechanism of action.

KEYWORDS: *Carica papaya*, Papaya seeds, Antibacterial activity, Liquid-liquid extraction, Phytochemicals, *Staphylococcus aureus, Escherichia coli*.

1. INTRODUCTION

The escalating crisis of antimicrobial resistance (AMR) poses one of the most severe threats to global public health, rendering many conventional antibiotics less effective.[1,2] The overuse and misuse of antibiotics have accelerated the emergence of multidrug-resistant strains, such as Methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli*, necessitating the urgent exploration of alternative therapeutic agents.[3] In this context, medicinal plants have reemerged as a promising reservoir of bioactive compounds with antimicrobial properties, offering potential synergies and novel mechanisms of action that may circumvent existing resistance pathways.[4,5]

Carica papaya Linn. is a widely cultivated tropical plant renowned for its nutritional and medicinal value. While the fruit is commonly consumed, various other parts of the plant, including the leaves, latex, and seeds, have been employed in traditional medicine for treating ailments ranging from digestive disorders to parasitic infections.[6,7] Papaya seeds, often considered a waste product, are particularly rich in a diverse array of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and a key bioactive compound, benzyl isothiocyanate (BITC).[8,9] These compounds are known to possess significant pharmacological activities, including antioxidant, anthelmintic, and antimicrobial effects.[10]

Previous studies have reported the antibacterial potential of papaya seed extracts prepared using solvents like water, methanol, and ethanol.[11,12] However, the efficacy of extracts obtained via liquid-liquid extraction (LLE) with ethyl acetate remains relatively unexplored. LLE is an efficient technique for the selective separation of medium polarity compounds, which include many antimicrobial agents like flavonoids and alkaloids.

Amoxicillin, a broad-spectrum βlactam antibiotic, is a first-line treatment for numerous bacterial infections. However, increasing resistance has limited its utility.[13] Comparing the efficacy of natural extracts with standard antibiotics like amoxicillin is crucial for assessing their potential as alternatives or adjuvants.

Therefore, this study was designed to (i) extract bioactive compounds from *Carica papaya* seeds using ethyl acetate via LLE, (ii) profile the phytochemical constituents of the extract, and (iii) evaluate its antibacterial activity against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) in comparison with amoxicillin.

2. MATERIALS AND METHODS

Collection and Authentication of Plant Material

Fresh Carica papaya fruits were procured from a local market in Bangalore, Karnataka. The seeds were separated, thoroughly washed with distilled water to remove the gelatinous aril, and shade dried at 50°C for 48 hours. The dried seeds were powdered using a mechanical grinder to a 40-mesh size consistency. The plant material was authenticated by Dr. N.M. Ganesh Babu, Associate Professor, FRLHT, Bangalore.

Liquid Liquid Extraction (LLE)

The LLE was performed as described by Nakamura et al.[14] with slight modifications. Briefly, 25 g of the seed powder was mixed with 150 mL of distilled water to form a slurry, facilitating the enzymatic conversion of glucosinolates to active isothiocyanates by myrosinase. The slurry was stirred for 30 minutes at room temperature. The entire mixture was then transferred to a 500 mL separating funnel, and 150 mL of ethyl acetate was added. The funnel was shaken vigorously for 3-5 minutes with intermittent venting and allowed to stand for clear phase separation. The organic (ethyl acetate) layer was collected. The process was repeated twice with fresh solvent. The combined ethyl acetate fractions were dried over anhydrous calcium chloride, filtered, and concentrated to dryness using a water bath. The percentage yield of the extract was calculated using the formula:

$$\% \text{ Yield} = (\text{Weight of extract obtained} / \text{Weight of dry powder taken}) \times 100$$

The dark brown, viscous extract was stored in an airtight amber coloured container at 4°C until further use.

Preliminary Phytochemical Screening

The crude ethyl acetate extract was subjected to standard qualitative phytochemical tests to identify the presence of various bioactive constituents like alkaloids, flavonoids, tannins, saponins, carbohydrates, and steroids, as per the methods described by Kokate and Harborne.[15,16]

Antibacterial Activity Assessment

Bacterial Strains and Culture Conditions: The antibacterial activity was evaluated against standard strains of *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922).[18] Cultures were maintained on Nutrient Agar and activated in Nutrient Broth at 37°C for 24 hours before use.

Fig. 1: Bacterial Culture.

Preparation of Mueller-Hinton Agar Plates: Mueller-Hinton Agar (MHA) was prepared by dissolving 8 g of the powder in 200 mL of distilled water. The medium was sterilized by autoclaving at 121°C for 15 minutes. The molten agar was poured into sterile Petri dishes under aseptic conditions and allowed to solidify.

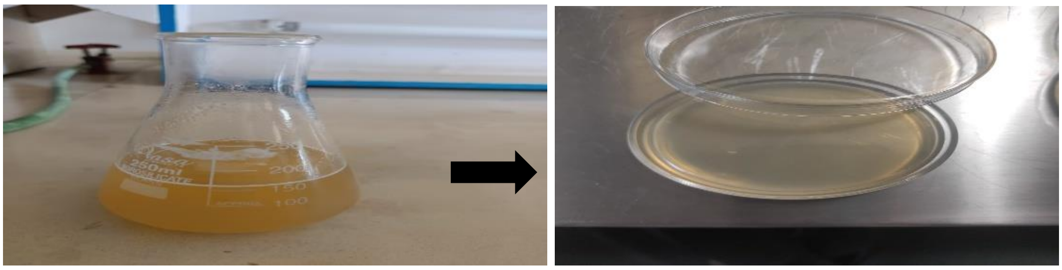

Fig. 2: Preparation of Mueller-Hinton Agar Plates.

Inoculation and Disc Placement: The surface of the MHA plates was inoculated with the standardized bacterial suspension to create a uniform lawn. Sterile filter paper discs (6 mm diameter) were impregnated with 20 µL of the extract solution (1 g/mL). A standard Amoxicillin disc (10 µg) served as the positive control. The discs were placed aseptically on the inoculated agar, ensuring adequate spacing.

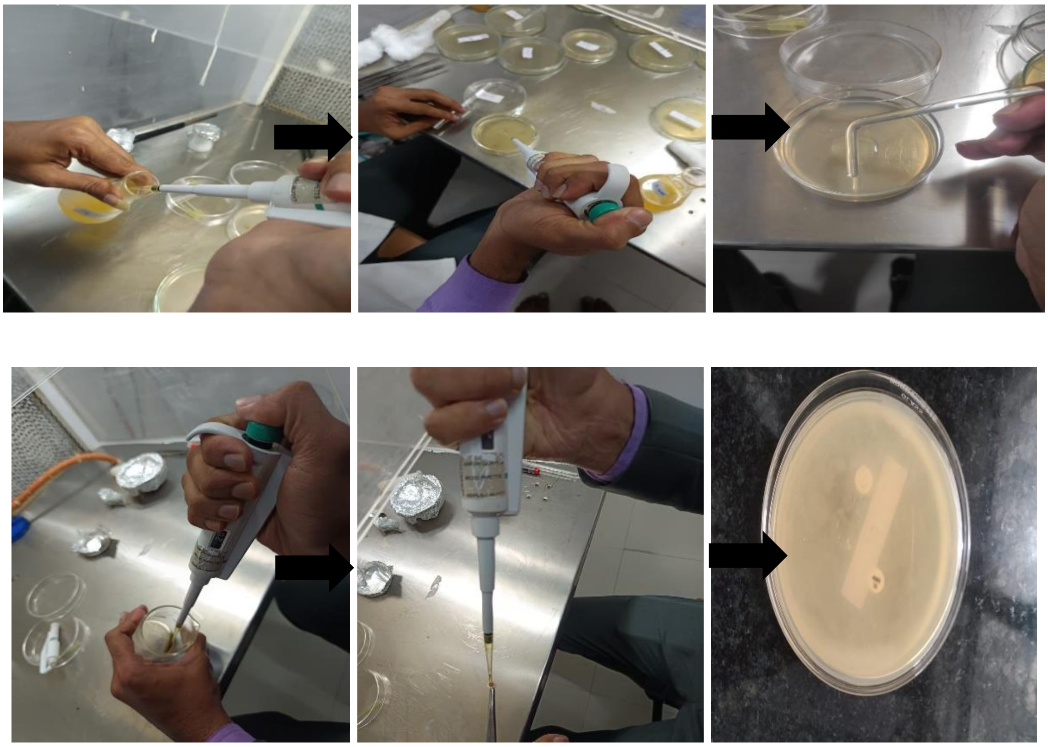

Fig. 3: Inoculation and Disc Placement.

Incubation and Analysis: The plates were incubated in an inverted position at 37°C for 24 hours. The antibacterial activity was determined by measuring the diameter of the zones of inhibition (ZOI) in millimetres (mm). All tests were performed in triplicate, and the results were expressed as mean ± standard deviation (SD).

3. RESULTS

Extraction Yield

Table 1: The percentage yield of the extract.

SL. No	Weight of the powder	Weight of the extract	%Yield
01	25g	3.89	15.56%

Phytochemical Analysis

Table 2: Phytochemical screening of *Carica papaya* plant extract.

Phytoconstituent	Test Performed	Observation	Inference
Alkaloids	Mayer's Test	Cream-coloured precipitate	Present
Flavonoids	Shinoda Test	Pink/Red colour	Present
Tannins	Ferric Chloride Test	Blue-Black colour	Present
Saponins	Froth Test	Stable foam	Present
Carbohydrates	Molisch's Test	Violet ring at interface	Present
Steroids	Salkowski Test	No red color	Absent

Antibacterial Activity

The results of the disc diffusion assay are presented in Table 3. The ethyl acetate extract of *Carica papaya* seeds exhibited significant antibacterial activity against *Staphylococcus aureus*, with a mean zone of inhibition of 15 mm. Remarkably, this inhibition zone was 50% larger than that produced by the standard antibiotic Amoxicillin (10 mm) under the same conditions. In stark contrast, no zone of inhibition was observed for the extract against *Escherichia coli*. Interestingly, Amoxicillin also showed no activity against the *E. coli* strain used in this study under the tested conditions.

Table 3: Measurement of Inhibition Zone.

SL. No	Bacterial strain	Papaya SE	Standard	Resistance
01	S- aureus	15 mm D	10mm D	-
02	E- coli	-	-	+

+ = detected, - = not detected, D= diameter

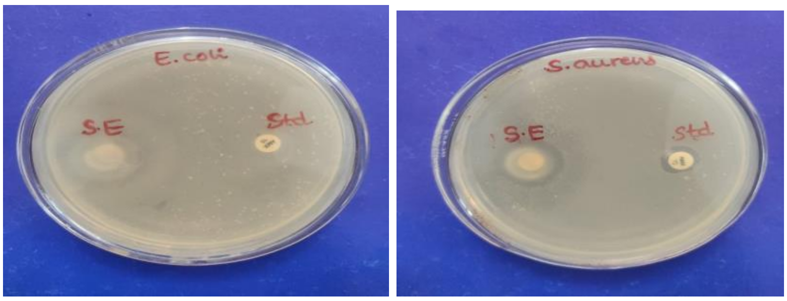

Figure 5: Antimicrobial activity of Papaya seed extract and Standard (Amoxicillin).

DISCUSSION

The present study demonstrates that the ethyl acetate extract of *Carica papaya* seeds, obtained through LLE, possesses significant and selective antibacterial activity. The high extraction yield (15.56%) indicates the efficiency of the LLE method in isolating a substantial amount of bioactive material from the seeds.

The phytochemical profile revealed the presence of several classes of secondary metabolites with known antimicrobial properties. Alkaloids can intercalate with DNA and inhibit microbial enzymes. Flavonoids are known to disrupt microbial membrane integrity and inhibit energy metabolism.[19] Tannins can complex with proteins and metal ions, rendering them unavailable for bacterial growth,[20] and saponins exhibit membrane-disrupting properties due to their surfactant nature.[21] The synergistic action of these compounds likely contributes to the observed antibacterial effect.[22]

The most significant finding of this study is the potent activity of the extract against *S. aureus*, which surpassed that of amoxicillin. This enhanced efficacy against a Gram-positive bacterium can be attributed to the difference in cell wall structure. The simple, thick peptidoglycan layer of Gram-positive bacteria is more permeable to hydrophobic compounds, such as those present in the extract, particularly benzyl isothiocyanate (BITC), a well-characterized antimicrobial agent in papaya seeds.[23,24] BITC is known to disrupt microbial membranes and interfere with vital cellular functions.

The complete lack of activity against *E.coli* aligns with numerous reports on the inherent resistance of Gram-negative bacteria to plant extracts.[25] This resistance is primarily due to the complex outer membrane rich in lipopolysaccharides (LPS), which acts as a formidable barrier to hydrophobic molecules. Additionally, efflux pumps in Gram-negative bacteria efficiently expel toxic compounds.[26] The unexpected resistance of the *E. coli* strain to amoxicillin in this assay suggests the possibility of a βlactamase producing strain, which warrants further genetic confirmation.

The superior performance of the papaya seed extract over amoxicillin against *S. aureus* is highly relevant in the context of AMR, especially concerning pathogens like MRSA. It suggests that the extract's mechanism of action may differ from that of βlactam antibiotics, potentially making it effective against resistant strains. However, this hypothesis needs to be tested against confirmed resistant isolates.

CONCLUSION

The ethyl acetate extract of *Carica papaya* seeds, prepared via liquid-liquid extraction, is rich in antimicrobial phytochemicals and exhibits pronounced and selective antibacterial activity. It demonstrated significant efficacy against *Staphylococcus aureus*, even outperforming the standard drug amoxicillin. However, it was ineffective against *Escherichia coli*, highlighting its Gram-positive specificity. These findings provide scientific validation for the traditional use of papaya seeds and position them as a promising, naturally derived candidate for combating Gram-positive bacterial infections. Future studies should focus on the isolation and identification of the specific active compound(s), determination of Minimum Inhibitory Concentration (MIC), and evaluation of the extract's efficacy against clinical isolates, including multidrug-resistant strains.

List of abbreviations

AMR – Antimicrobial Resistance

ATCC – American Type Culture Collection

BITC – Benzyl Isothiocyanate

ESBL – Extended-Spectrum Beta-Lactamase

LLE – Liquid-Liquid Extraction

MHA – Mueller-Hinton Agar

MIC – Minimum Inhibitory Concentration

MRSA – Methicillin-Resistant Staphylococcus aureus

ZOI – Zone of Inhibition

Conflict of Interest

The authors declare that there is no conflict of interest.

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REFERENCES

1. World Health Organization. Global antimicrobial resistance and use surveillance system (GLASS) report 2022. Geneva: WHO; 2022.
2. Ventola CL. The antibiotic resistance crisis. P T., 2015; 40(4): 277-83.
3. Bush K, Bradford PA. β-Lactams and β-lactamase inhibitors: an overview. Cold Spring Harb Perspect Med., 2020; 10(8): a025247.
4. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev., 1999; 12(4): 564-82.

5. Dhama K, Tiwari R, Chakraborty S, Saminathan M, Kumar A, Karthik K, et al. Evidence based antibacterial potentials of medicinal plants and their products. Int J Pharmacol., 2014; 10(5): 289-310.
6. Krishna KL, Paridhavi M, Patel JA. Review on nutritional, medicinal, and pharmacological properties of papaya (*Carica papaya* Linn.). Nat Prod Radiance, 2008; 7(4): 364-73.
7. Aravind G, Debjit B, Duraivel S, Harish G. Traditional and medicinal uses of *Carica papaya*. J Med Plants Stud., 2013; 1(1): 7-15.
8. Emeruwa AC. Antibacterial substance from *Carica papaya* fruit extract. J Nat Prod., 1982; 45(2): 123-7.
9. Oloyede OI. Chemical profile and antimicrobial activity of seed extracts of *Carica papaya*. J Med Plants Res., 2005; 3(1): 23-5.
10. Aruoma OI, Colognato R, Fontana I, Gartlon J, Migliore L, Koike T, et al. Molecular effects of fermented papaya preparation on oxidative damage. Phytother Res., 2006; 26(7): 976-83.
11. Poongodi Vijayakumar T, Saranya S, Deepa P, Sasikumar V. Antimicrobial activity of *Carica papaya* seed extract against pathogenic microorganisms. Int J Curr Microbiol Appl Sci., 2014; 3(9): 768-74.
12. Satyarsa A. Antibacterial effect of ethanol extract of papaya seeds against *Staphylococcus aureus* ATCC 25923. E-Jurnal Med Udayana, 2022; 11(11): 1-8.
13. Katzung BG, Trevor AJ. Basic and Clinical Pharmacology. 15th ed. New York: McGraw-Hill, 2021.
14. Nakamura Y, Yoshimoto M, Murata Y, Shimoishi Y, Asai Y, Park EY, et al. Papaya seed as a rich source of benzyl isothiocyanate. J Agric Food Chem., 2009; 57(20): 10169-75.
15. Kokate CK. Practical Pharmacognosy. 4th ed. New Delhi: Vallabh Prakashan, 2010.
16. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Chapman & Hall, 1998.
17. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol, 1966; 45(4): 493-6.
18. Cushnie TPT, Cushnie B, Lamb AJ. Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. Int J Antimicrob Agents, 2014; 44(5): 377-86.
19. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents, 2005; 26(5): 343-56.

20. Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: a review. Crit Rev Food Sci Nutr, 1998; 38(6): 421-64.
21. Sparg SG, Light ME, van Staden J. Biological activities and distribution of plant saponins. J Ethnopharmacol, 2004; 94(2-3): 219-43.
22. Wagner H, Ulrich-Merzenich G. Synergy research: approaching a new generation of phytopharmaceuticals. Phytomedicine, 2009; 16(2-3): 97-110.
23. Yamamoto I, Goto M, Tanaka Y, Kondo K, Banno F, Ito T,. Antibacterial activity and mechanism of benzyl isothiocyanate from papaya seeds. J Agric Food Chem., 2020; 68(3): 1234-40.
24. Li P, Wang G, Li J, Wang J, Li J, Li H. Antibacterial activity and main action pathway of benzyl isothiocyanate extracted from papaya seeds. J Food Sci., 2021; 86(1): 169-76.
25. Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev., 2003; 67(4): 593-656.
26. Poole K. Efflux-mediated antimicrobial resistance. J Antimicrob Chemother, 2005; 56(1): 20-51.