

Research Article

Phytochemical Analysis and Antimicrobial Activity of *Carica Papaya* Seed and Bark Against Selected Pathogens, in Case of Southern Ethiopia

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Abstract

The phytochemical constitution and antibacterial properties of *Carica Papaya* seeds and bark that have been collected from the West Guji district in Southern Ethiopia are examined in this study. This investigation aims to explore the traditional medicinal significance of the plant by analyzing its chemical makeup and biological activities. The study uses three extraction solvents methanol, petroleum ether, and ethyl acetate with the goal of identifying bioactive chemicals and evaluating their effectiveness against particular infections. These solvents were selected to ensure the extraction of a broad range of polar and non-polar compounds. Alkaloids, flavonoids, saponins, tannins, terpenoids, glycosides, steroids, and phenolic chemicals were found in a variety of extracts using phytochemical screening, indicating a rich profile of secondary metabolites that are often associated with antimicrobial and therapeutic effects. The antifungal activity against *Candida albicans* and *Aspergillus niger* was assessed using broth dilution techniques, which allowed for the determination of minimum inhibitory concentrations, while the antibacterial activity against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) was assessed using the agar diffusion method, a standard approach to evaluate the inhibition zone diameter. With inhibitory zones that measured 12 mm for *E. coli* and 7 mm for *S. aureus*, the methanol extract of seeds had the strongest antibacterial efficacy, suggesting that polar phytochemicals present in methanol extracts may be particularly effective. These results demonstrate the therapeutic potential of *C. papaya* as a natural source of antibacterial agents and the necessity of more research into its bioactive components for possible medical uses, including drug development, formulation of alternative therapies, and its integration into natural health products.

Keywords

Phytochemicals, Antimicrobial Activity, Inhibitory Zones, *Carica Papaya*, Extraction Methods, Therapeutic Potential

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1. Introduction

Background of the Study

Plants have not only nutritional value but also, in the eyes of the local people, they have medicinal and ritual or magical values. Traditional medicinal plants have important contributions in the health care system of local communities as the main source of medicine for the majority of the rural population. These medical systems are heavily dependent on various plant species and plant based products [1, 2].

Phytochemical screening of plants has revealed the presence of numerous chemicals including alkaloids, flavonoids, Steroids, terpenoids, saponins, tannins, Phenols and glycosides. The medicinal values of some plants lie in these chemical substances that produce definite physiological actions in the human body. Many of these indigenous medicinal plants are used as spices and food plants [3]. Plant serves as rich resources of natural drugs for research and development. The beneficial medicinal effects of plant materials typically result from the combinations of secondary metabolites present in plants. The medicinal actions of plants are unique to plant species or groups as the combination of secondary products in a plant are often taxonomically distinct [4]. Secondary metabolites of plants serve as defense mechanisms against predation by many microorganisms, insects and herbivores [5].

Microbial pathogenicity and other infectious disease has been prevalence throughout the world. Due to that reason worldwide population was suffer from such microbial pathogens, so the worldwide society began to fight against these infectious diseases by creating (providing) medicines, which are extracted from the plants which found in its surrounding [5].

The antimicrobial activity of the spices is due to specific phytochemical. The main factors that determine the antimicrobial activity are the type and composition of the species (medicinal plant) among them. *Carica Papaya* L is the natural herbs' act as tonic to keep your health fit and sound. This has a large scale of mutational value which have made them unified, effective as well as more power full for good health. Several trials and modern sciences have been confirmed that *Carica Papaya* can be used as herbal medicine and should be used (consumed) daily at a particular amount [6].

The fruit of the *Carica Papaya* plant contains a chemical called capsaicin. Capsaicin seems to reduce pain sensations when applied to the skin. A particular form of capsicum causes intense eye pain and other unpleasant effects when it comes in contact with the face. This form is used in self-defense pepper sprays [6].

Depending on its diver's flora Ethiopia was ranking fifth largest flora in Africa [7]. The increasing prevalence of antimicrobial resistance among pathogenic microorganisms poses a significant challenge to public health, particularly in developing regions such as Southern Ethiopia. Traditional medicinal plants have been recognized for their potential therapeutic properties, including antimicrobial activity. *Carica Papaya*, commonly known for its nutritional benefits, has

been underexplored for its phytochemical constituents and their efficacy against various pathogens [8].

Despite the traditional use of papaya seeds and bark in folk medicine, there is a scarcity of scientific data validating their antimicrobial properties. The seeds and bark of *Carica Papaya* contain diverse phytochemicals, including alkaloids, flavonoids, and tannins, which could contribute to their antibacterial and antifungal activities. However, systematic investigations into the phytochemical profile and the antimicrobial potential of these plant parts against specific pathogens prevalent in Southern Ethiopia are lacking [9, 10].

The absence of comprehensive studies limits the understanding of the therapeutic potential of *Carica Papaya* and its derivatives, which could serve as alternative or complementary treatments in managing infections caused by resistant pathogens. Therefore, this research aims to fill the knowledge gap by conducting a thorough phytochemical analysis of *Carica Papaya* seeds and bark, followed by an evaluation of their antimicrobial activity against selected pathogens. This study will not only contribute to the scientific understanding of *Carica Papaya* but also potentially offer new avenues for developing effective antimicrobial agents in the fight against drug-resistant infections in Southern Ethiopia.

The significance of this study on the phytochemical analysis and antimicrobial activity of *Carica Papaya* seeds and bark against selected pathogens in Southern Ethiopia can be used addressing antimicrobial resistance, phytochemical insights, cultural relevance and investigating the antimicrobial properties of *Carica Papaya* aligns with local practices and beliefs in Southern Ethiopia, where traditional medicine plays a significant role in healthcare [9-11]. This research can validate and promote the use of local medicinal plants, fostering a sense of cultural pride and encouraging sustainable practices in herbal medicine.

The study promotes the sustainable use of local plant resources, which can help in conserving biodiversity while providing health benefits to the community. It emphasizes the importance of integrating traditional knowledge with scientific research to enhance healthcare outcomes [11]. In summary, this study not only aims to validate the traditional uses of *Carica Papaya* but also seeks to provide scientific evidence that could lead to practical applications in combating infectious diseases in Southern Ethiopia.

2. Materials and Methods

2.1. Description of the Study Area

The study was conducted in Bule Hora University Southern Oromia Region, Ethiopia. Located on the paved Addis Ababa-Moyale highway, in the West Guji Zone, It is located in latitude and longitude of 5°35'N 38°15'E and an altitude of 1716 meters above sea level and for some characterization

another university like Addis Ababa science and Technology University and Arba Minch University were used.

2.1.1. Sample Site

Carica Papaya plant seed and bark were collected from Tulage and Bule Irsha kebele, in West Guji Zone Bule Hora woredas located in Southern of Oromia Regional State which is 465 km away from Addis Ababa, the Capital city of the country Ethiopia and the water aqueous solution was prepared in Bule Hora University Chemistry laboratory.

2.1.2. Experimental Site

The experiments were conducted in Bule Hora University Chemical Engineering and Chemistry laboratory and Abaya campus Arba Minch University.

2.2. Materials

2.2.1. Chemicals and Reagents

The major chemicals and reagents were used for this study were: Potassium Iodide, Sodium, distilled water, sulphuric acid, hydrochloric acid, acetic acid, acetic anhydride, glacial acetic acid, Ferric chloride, magnesium, sodium hydroxide, potassium hydroxide, lead acetate, copper Sulphate.

2.2.2. Apparatus and Instruments

The following apparatus was used: electrical grinder, rotary evaporator (RE200) filter paper, refrigerator, electronic balance, Hot plate, autoclave, Petridishes, ovens, refrigerator and incubator.

2.2.3. Study Design

For this research study, Papaya (*Carica Papaya*) plant was selected. The selected two parts of Papaya (Bark and seeds) were obtained from Tulage and Bule Irsha kebele, in West Guji Zone Bule Hora woredas and extracted using three extraction solvents (methanol, petroleum ether and Ethyl acetate). Although, for the study of anti-microbial activities two microorganisms were used for the antimicrobial assays. Antimicrobial assays were carried out for each extract in triplicate.

2.2.4. Collection of Plant Material

The fresh seed and bark of the plant *Carica Papaya* was collected from Tulage and Bule Irsha, Bule Hora woredas. The collected seed and bark of *Carica Papaya* was taken in a plastic bag and immediately brought to the laboratory.

2.3. Pathogens Culture

Human pathogens *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) bacteria were used for the research. The micro-organism isolated are include one strain of Gram negative bacteria *E. coli* and gram positive bacteria *S.*

aureus was cultured in laboratory and is maintained on Mueller Hinton Agar. They are kept in the refrigerator set at 4 °C up to they used for the experimental application [12].

2.4. Sample Preparation and Size Determination

2.4.1. Seed

The collected fresh seeds were extracted from collected ripe papaya fruits. The fruits were cut open to extract the seeds and it was washed with tap water, rinse in sterile distilled water and dried for 21 days at room temperature. The dried sample was powdered using grinder and powdered seed of *Carica Papaya* were prepared. The finely powdered seed sample were prepared without any impurities mixed in them was packed. Then they were stored in the laboratory room at room temperature up to it was used for extraction [13].

2.4.2. Bark

Disease free bark of papaya was cut from a tree using a sharp kitchen knife. They are washed in tap water and rinsed in sterile distilled water then dried under room temperature for 21 days. [14]. The dried sample was powdered using grinder and powdered bark of *Carica Papaya* were prepared. The finely powdered bark sample were prepared without any impurities mixed in them was packed. Up to it was taken for the extraction purpose they were stored at the laboratory room at room temperature [13].

2.5. Extraction of Plant Material by Organic Solvent

Totally three types of extracts are collected using three different organic solvents, petroleum ether, ethyl acetate and methanol solvents were used for extraction. The collected extracts from seeds and barks of the papaya plant samples are done by three solvents from individual plant materials.

2.5.1. Methanol Extraction of Seed and Bark

75g grams finely powdered seeds and bark sample were weighed separately using an electronic weighing balance and were macerated with 600 ml of 95% methanol in different round bottom flasks separately. The macerated solution of both seed and bark powder sample were placed at room temperature and shaken occasionally for 48 hours. After 48 hours the extracts was filtered using what man No. 1 filter paper. The percentage yield of the extract were determined based on the sample powdered weight [15].

$$\text{Percentage yield of samples} = \frac{\text{Weight of oil extract} \times 100}{\text{Weight of sample powder}}$$

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$$\text{Percentage yield of seed} = \frac{10.75 \text{ g} \times 100}{75 \text{ g}} = 14.33\%$$

$$\text{Percentage yield of bark} = \frac{9.50 \text{ g} \times 100}{75 \text{ g}} = 12.66\%$$

2.5.2. Petroleum Ether Extraction of Seed and Bark

The finely powdered seed and bark stored in lab prepared samples of each 75 g was macerated with 600 mL of petroleum ether in different round bottom flask separately. Then it was shaken occasionally for 48 hours. Then the extracts were filtered using Whatman No. 1 filter paper. The percentage yield of the extract was determined based on the sample powdered weight [16].

$$\text{Percentage yield} = \frac{\text{Weight of oil extract} \times 100}{\text{Weight of sample powder}}$$

$$\text{Percentage yield of seed} = \frac{11.5 \text{ g} \times 100}{75 \text{ g}} = 15.33\%$$

$$\text{Percentage yield of bark} = \frac{10.25 \text{ g} \times 100}{75 \text{ g}} = 13.66\%$$

2.5.3. Ethyl Acetate Extraction of Seed and Bark

Extraction by ethyl acetate was done in the similar way of the methanol and petroleum ether procedures. The percentage yield of the crude extract is determined based on the sample powdered weight below [16].

$$\text{Percentage yield} = \frac{\text{Weight of oil extract} \times 100}{\text{Weight of sample powder}}$$

$$\text{Percentage yield of seed} = \frac{13 \text{ g} \times 100}{75 \text{ g}} = 17.33\%$$

$$\text{Percentage yield of bark} = \frac{11 \text{ g} \times 100}{75 \text{ g}} = 14.66\%$$

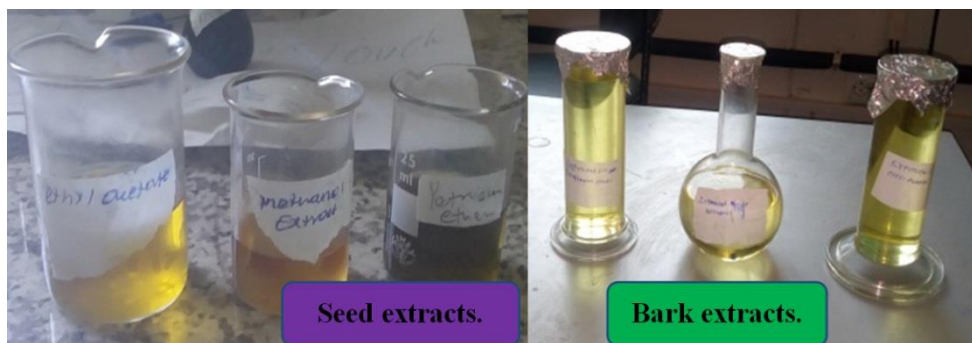


Figure 1. Seed and bark extracts by three organic solvents.

2.6. Preliminary Phytochemical Screening Test

Phytochemical screenings are preliminary tests used to detect the presence of primary and secondary metabolites in an extract. This study focuses on qualitative phytochemical screening of *Carica Papaya* seed and bark extract to identify secondary metabolite compounds. Standard procedures and three samples were used for each test to confirm the presence or absence of secondary metabolites. The plant extract, seed and bark of *Carica Papaya* containing various bioactive components with varying reducing capacities, led to the anti-microbial activity [17].

Preliminary screening were tested for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compound, flavonoids, saponins, tannins and cardiac glycosides. The following bioactive tests has been performed to confirm the presence or absence of the secondary metabolites in the seed and bark extracts.

For phytochemicals investigation study the prepared finely powdered sample of seed and bark were dissolved in the

solvents and a stock solution of 5g/100ml for both samples in triplets was made. This was done by mixing 5gm of milled *Carica Papaya* seed and bark were mixed in three different glass flask by triplets of both with 100 ml of distilled water and it was filtered by using Whatman No. 1 filter paper. All three stock solution was made by this way.

(i) Test for Flavonoids

Shinoda test - 2 ml of each extracts the petroleum ether, ethyl acetate and methanol seed extract for each sample was taken in different test tube and each of them were mixed with few fragments of magnesium turnings and concentrated HCl was added drop wise. Appearance of pink scarlet/ reddish coloration after few minutes indicates the presence of flavonoids. The procedure were repeated for bark extracts [18, 19].

(ii) Test for Alkaloids

Like flavonoids are checked 5 ml of petroleum ether, ethyl acetate and methanol seed extract was taken in different test tube and evaporated to dryness. Residues were heated on a boiling water bath with 2%HCL. The filtered and treated with Mayer's reagent. Yellow precipitate indicating the presence of

alkaloid. The procedure were repeated for bark extracts [19-21].

(iii) Test for Terpenoids

Salkowski test- 5 ml of each extract were mixed with 2 ml of chloroform and 3 ml of sulphuric acid were added from the sides of the tube in different three test tubes which have petroleum ether, ethyl acetate and methanol seed extract. Formation of reddish-brown coloration at interface indicated the presence of terpenoids. The procedure were repeated for bark extracts [19].

(iv) Test for Steroids

Liebermann Burchard's test- petroleum ether, ethyl acetate and methanol seed extract were dissolved in 2 ml of acetic anhydride and 1-2 drops of sulphuric acid were added along sides of the test tube in different test tube. Blue green ring appears or the array of color changes indicates the presence of steroids. The procedure were repeated for bark extracts [21, 22].

(v) Test for Tannins

To the crude petroleum ether, ethyl acetate and methanol seed extract were taken in clean another different test tube, add 2 drops of FeCl_3 . Formation of dark green colour indicates the presence of tannin. The procedure were repeated for bark extracts [19].

(vi) Test for Saponins

Froth test- 5ml of distilled water were mixed with 1 ml of crude petroleum ether, ethyl acetate and methanol seed extract in the different test tube and were shaken vigorously. The formation of stable foam is taken as an indication for the presence of saponins. The procedure were repeated for bark extracts [21].

(vii) Test for Glycosides

To 1 gm. of each seed extract in different test tube, 5 ml of distilled water were added to dilute the sample adds 2 ml of glacial acetic acid were added and containing one drop of ferric chloride solution. This was underplayed with 1 ml of conc. H_2SO_4 . Brown ring at the interface indicates the presence of glycosides. The procedure were repeated for bark extracts [22].

(viii) Test for Phenolic Flavonoids:

1ml of each three of the each seed extracts were mixed with 2 ml of 10% lead acetate in different dry test tube and the formation of brown precipitate is taken as an indication for the presence of phenolic compounds. The procedure were repeated for bark extracts [18-21].

2.7. Antimicrobial Activities of Papaya Seed and Barks Extracts

2.7.1. Antibacterial Activity Test

For test of antibacterial activities of the extracts the well diffusion method was used. A positive and negative microbial control was used to compare the results. Antimicrobial susceptibility test measures the ability of an antimicrobial agent to inhibit bacterial growth. In this study the antimicrobial activities of the oil extract of three organic solvents such as petroleum ether, ethyl acetate and methanol of the seed and bark of papaya were done on *S. aureus* as gram positive bacteria and *E. coli* as gram negative bacteria. The test was performed in triplicates.

2.7.2. Collection of Test Organism

Human pathogens *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) used in this study were taken from biotechnology Laboratory of Arba Minch University Abaya campus.

2.7.3. Preparation of Media

Muller-Hinton agar preparation

8g of Mueller-Hinton agar (MHA) powder was dissolved in 250ml of distilled water, then heated, Shacked well and allowed to boil. It was then put in autoclave at 121 °C for about 15 minutes to sterilize the media.

The media were allowed to cool and pour in eight plates and put on leveled surface.

The media were allowed to solidify, kept in the upright in the incubator avoiding contamination from the hood.

Micro-organisms used for the test were *streptococcus* and *Escherichia Coli* bacteria as gram -positive and Gram-negative bacteria, respectively.

2.7.4. Zone of Inhibition

The zone of inhibition is determined using the nutrient agar method. By using the petri-dishes with each petri-dish corresponding to one test organism for each extracts are well labelled and used. The nutrient agar will be allowed to solidify and wells created in them using the cork borer (6 mm) with each well filled with its respective concentration of the plant extracts and left for about 1 hour for complete diffusion of the extract within the nutrient agar. The petri-dishes containing the nutrient agar are then incubated between 37 °C and 42 °C for a period of 24 hours after which the zone of inhibition will be determined [23].

$$\text{Inhibition (\%)} = \frac{\text{Weight of oil} \times \text{Growth of microbes in Control (mm)} - \text{Growth of microbes in Extract (mm)}}{\text{Growth of microbes in Control (mm)}}$$

3. Result and Discussion

The phytochemical analysis of the methanol, petroleum

ether, and ethyl acetate extracts from the seeds and stem bark of *Carica Papaya* reveals a diverse array of secondary metabolites, which are crucial for the plant's potential therapeutic applications. The results presented in Table 1 highlight the presence of various phytochemicals, including tannins, sapo-

nins, alkaloids, flavonoids, terpenoids, glycosides, steroids, and phenols, indicating the rich bioactive profile of this plant.

Methanol Extracts: The methanol extracts from both seeds and stem bark exhibited a wide range of phytochemicals. Notably, compounds such as tannins, saponins, flavonoids, terpenoids, glycosides, and phenols were present in the seed extracts, suggesting their potential health benefits, including antioxidant and antimicrobial properties. The presence of alkaloids in the seeds further supports their medicinal value, as these compounds are often associated with various pharmacological activities.

Petroleum Ether and Ethyl Acetate Extracts: The petroleum ether extracts showed a different profile, with active components like alkaloids and steroids found in both seeds and stem

bark. This suggests that non-polar solvents can effectively extract certain phytochemicals, contributing to the diverse therapeutic potential of *C. papaya*. However, the ethyl acetate extracts were less comprehensive, with fewer compounds detected, indicating that the solvent choice significantly influences the extraction efficiency of specific phytochemicals.

Variability between Plant Parts: The variability in phytochemical composition between the seeds and stem bark is noteworthy. The stem bark was generally less rich in certain compounds, such as tannins and flavonoids, which were predominantly found in the seed extracts. This suggests that different parts of the plant may serve distinct roles in traditional medicine and could be targeted for specific therapeutic applications.

Table 1. Phytochemical analysis of methanol, petroleum ether and ethyl acetate extracts from seed and stem bark of *Carica Papaya* plant parts.

Phytochemical components	Methanol extract		petroleum ether extract		ethyl acetate extract	
	Seed	Stem bark	Seed	Stem bark	Seed	Stem bark
Tannins	+	-	-	+	-	-
Saponins	+	-	-	+	-	-
Alkaloids	+	-	+	-	+	+
Flavonoids	+	+	+	-	-	-
Terpenoids	+	+	+	+	+	+
Glycoside	+	+	+	-	-	+
Steroids	-	+	+	+	-	-
Phenols	+	+	+	-	+	-

Table 1 indicated the screening of different secondary metabolite compounds of *Carica Papaya*. This data confirmed the findings of other researchers where these compounds showed antimicrobials activities [24].

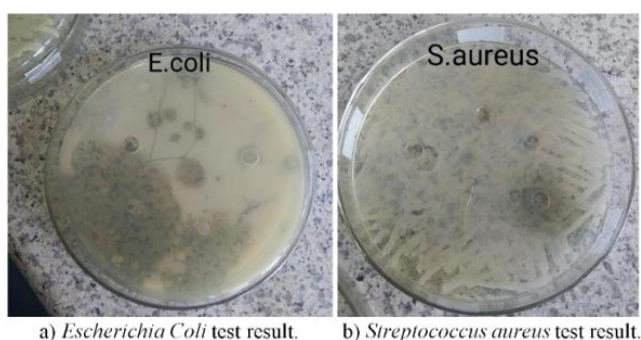


Figure 2. Antimicrobial activities of seed and bark.

The results from the antibacterial activity tests of *Carica*

Papaya seed and bark extracts indicate that the plant possesses significant antibacterial properties, particularly in its seed extracts. The agar diffusion method employed in this study allowed for a clear demonstration of the zones of inhibition against the tested bacterial strains, *Escherichia coli* and *Staphylococcus aureus*.

Effectiveness of Seed Extracts: The methanol extract of the seeds showed the highest antibacterial activity, with *E. coli* presenting a zone of inhibition of 12 mm and *Staphylococcus aureus* showing 7 mm. These results suggest that the seed extracts contain potent antibacterial compounds, making them a promising candidate for further exploration as natural antibacterial agents. The effectiveness of the methanol extract aligns with previous studies reporting that methanol is an efficient solvent for extracting bioactive compounds, enhancing their antibacterial properties.

Comparison of Extract Types: The results indicated that the methanol extracts consistently outperformed petroleum ether and ethyl acetate extracts in terms of antibacterial activity. For instance, the petroleum ether extract of the seeds showed a

zone of inhibition of only 9 mm against *E. coli*, while the ethyl acetate extract yielded a slightly better result of 10.5 mm. This suggests that polar solvents like methanol may be more effective in extracting the compounds responsible for antibacterial activity.

Limited Activity of Bark Extracts: The antibacterial activity of the bark extracts was notably lower than that of the seeds. The highest inhibition zone for the bark extracts was recorded at 6 mm for *E. coli* with the methanol extract, while no inhibition was observed for *Staphylococcus aureus* with petroleum ether and ethyl acetate extracts. This discrepancy may be attributed to the different phytochemical compositions between the seed and bark, indicating that the seeds are more enriched with antibacterial compounds.

Clinical Implications: The observed antibacterial activity, especially against *E. coli*, suggests potential applications in treating infections caused by this bacterium. Given the rising concern over antibiotic resistance, utilizing natural extracts like those from *Carica Papaya* could offer alternative therapeutic options.

4. Conclusion

The phytochemical screening of methanol, petroleum ether, and ethyl acetate extracts from the seeds and stem bark of *Carica Papaya* demonstrates the plant's rich bioactive profile, with various secondary metabolites identified. The results indicate that *C. papaya* possesses significant potential for use in herbal medicine, particularly due to the presence of compounds known for their antioxidant, antimicrobial, and anti-inflammatory properties.

Future studies should focus on isolating and characterizing these bioactive compounds to better understand their mechanisms of action and therapeutic efficacy. Additionally, exploring the potential synergistic effects of these metabolites could enhance the development of natural remedies derived from *Carica Papaya*. Overall, this research underscores the importance of *C. papaya* as a valuable resource in the field of pharmacognosy and natural product development.

The antibacterial activity tests conducted on methanol, petroleum ether, and ethyl acetate extracts of *Carica Papaya* seeds and bark reveal significant potential for these extracts as natural antibacterial agents. The seed extracts, particularly those obtained using methanol, demonstrated the most substantial activity against *Escherichia coli* and *Staphylococcus aureus*, indicating that they are a rich source of bioactive compounds.

These findings support the traditional use of *C. papaya* in herbal medicine and highlight its potential for developing alternative treatments for bacterial infections. Future research should focus on isolating the specific antibacterial compounds within these extracts and examining their mechanisms of action, as well as exploring the potential for formulating these extracts into effective therapeutic products. Overall, *Carica Papaya* represents a promising area for further investigation in the quest for natural antibacterial solutions.

Abbreviations

E. Coli	Escherichia Coli
MHA	Mueller-Hinton Agar
S. aureus	Staphylococcus Aureus

Acknowledgments

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Author Contributions

Gemechu Duguma Argessa: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Writing – original draft, Writing – review & editing

Sena Megersa: Conceptualization, Investigation, Supervision, Project administration

Wondi Kabato: Conceptualization, Investigation, Methodology, Project administration

Wabi Reggasa: Conceptualization, Formal Analysis, Validation, Supervision, Writing – review & editing

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article. Additional datasets, if required, are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

This study, titled "Phytochemical Analysis and Antimicrobial Activity of *Carica Papaya* seed and Bark against selected pathogens, in Case of Southern Ethiopia" was conducted in compliance with ethical research standards. No human participants, human data, or human tissue were involved in this research. The experimental protocols for plant-based studies were carried out in accordance with institutional and national guidelines for agricultural research. Permission for field experimentation was obtained from the relevant local authorities and agricultural institutions.

Consent to Publish

The authors affirm that the manuscript contains original research findings and has not been published or submitted

elsewhere. All authors have reviewed the final version of the manuscript and provided their consent for publication in the Journal of Agriculture by Springer Nature. Any necessary permissions for data, images, or third-party materials have been obtained and are appropriately cited.

Clinical Trial

The research was No human participants, human data, or human tissue were involved in this research. Therefore, Clinical trial number is not applicable.

Appendix

Funding

This research was financially supported by Bule Hora University. The funding enabled the procurement of necessary materials, laboratory facilities, and analytical tools required for the successful execution of this study.

Conflicts of Interest

The authors declare no conflicts of interest.



Figure 3. Extraction of seed and stem bark of *Carica Papaya* for phytochemical analysis.

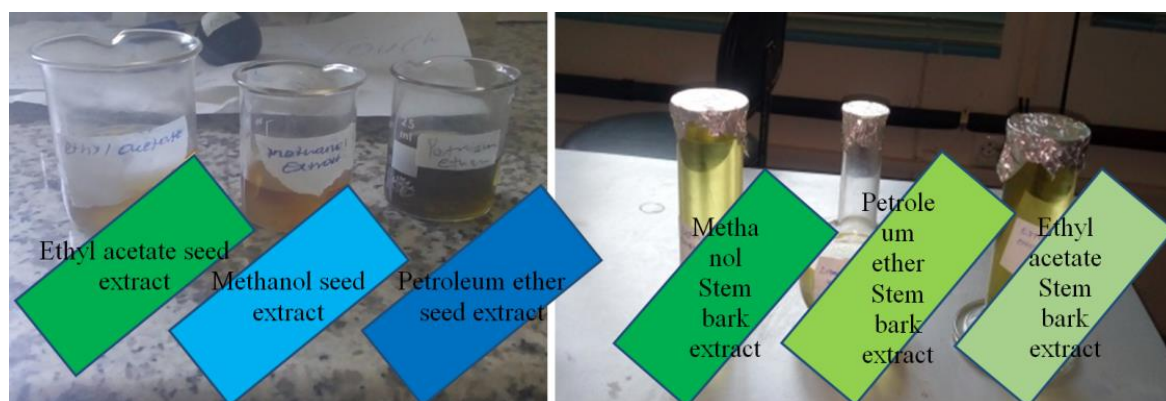


Figure 4. Extracted papaya seed and stem bark oil.

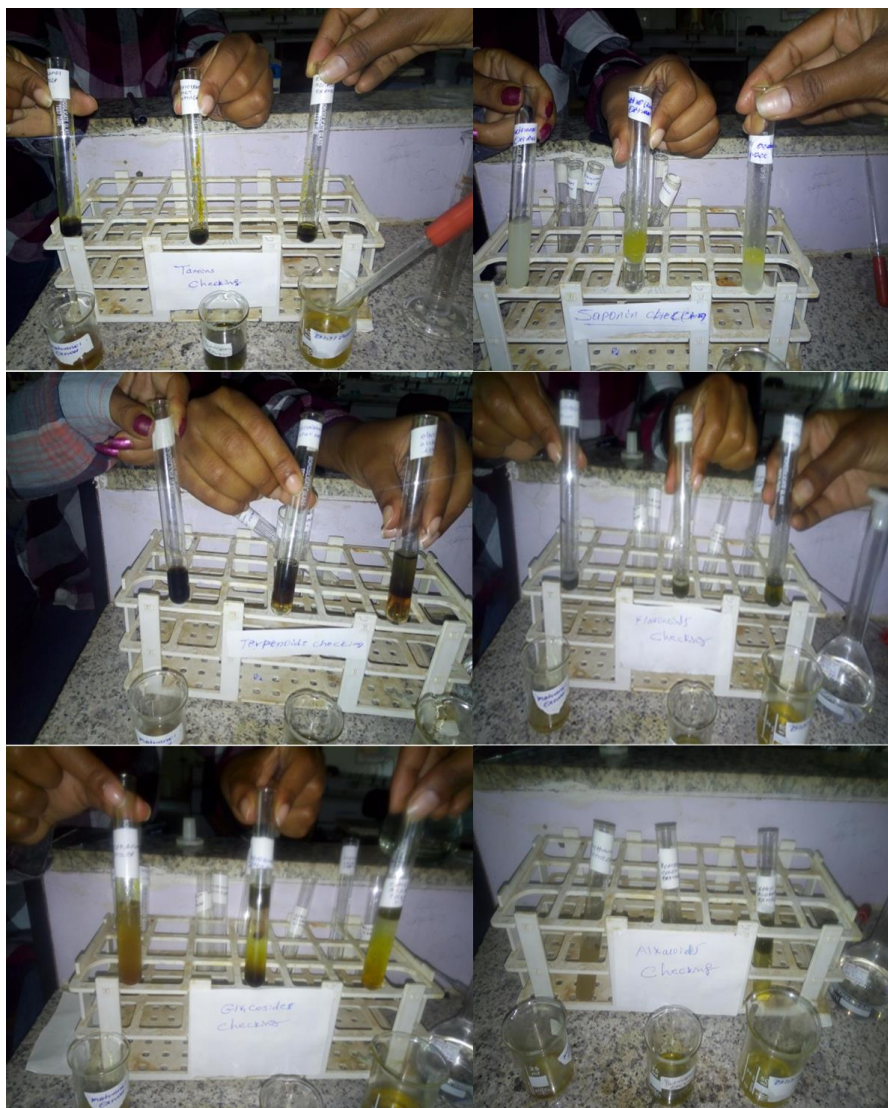


Figure 5. Phytochemical Analysis.

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