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Antibacterial Activity of Fermenting Unripe Pawpaw Parts (*Carica papaya*) against Some Enteric Bacteria

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Authors' contributions

This work was carried out in collaboration among all authors. Author BTF designed the study, performed the statistical analysis, managed the literature searches, wrote the protocol and wrote the first draft of the manuscript. Author OVO designed the study with author BTF wrote the protocol and managed the analysis, while author ATA managed the analysis and also assisted in editing the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

This research work was carried out to investigate the antibacterial activity of fermenting *Carica papaya* parts on some enteric organisms and their proximate and phytochemical screening. The unripe *Carica papaya* parts used were obtained from the Federal University of Technology Akure and the test organisms used are clinical isolates gotten from State Specialist Hospital, Akure. The unripe *Carica papaya* was separated into the leaves, peel, pulp and seed. It was then soaked in distilled water in separate fermenting jars for seven days. The following readings were taken daily; pH, titratable acidity while the Antibacterial effect of the fermenting slurry was monitored on day 1, 3, 5 and 7, using the agar well diffusion method. The result obtained showed that the pH decreases while the titratable acidity increases daily respectively. The zones of inhibition observed in the isolates were highest on day 3 and day 5, showing that the water from the fermenting unripe *Carica papaya* is more effective against the enteric isolates used for this research on day 3 and day 5. Therefore, water from fermenting unripe *Carica papaya* parts may be effective in treating infections from enteric organisms on day 3 and day 5. The peel and pulp were observed to be more effective while the seed had no antibacterial activity. Phytochemical analysis showed that the plant parts contained these active ingredients at various concentrations; alkaloids, tannins, saponins, glycosides and phenols.

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1. INTRODUCTION

Enteric bacteria are bacteria in the family Enterobacteriaceae. All members of this family possess these characteristics in common: gram-negative, rod-shaped, facultatively anaerobic, positive for true catalase and cytochromes, ferment glucose by one of two major pathways to a variety of end products, oxidase-negative, possess the Enterobacterial common antigen in the cell wall. These bacteria reside normally in the guts of many animals, including humans, and some are pathogenic, causing disease in certain animal species, examples include *Salmonella*, *Proteus*, *Serratia*, *Enterobacter*, *Citrobacter*, *Pseudomonas*, and *Klebsiella*. Infectious disease is the number one cause of death accounting for approximately one-half of all deaths in tropical countries. People usually become infected with enteric bacteria as a result of poor hygiene and contact with people who have existing infections. Some enteric bacteria can be controlled with the use of antibiotics and other drugs which attack the bacteria in the gut. The search for antimicrobial substances continues unabated, and with continuing fruitful result. These antimicrobial agents are agents that kill or inhibit the growth of microorganisms [1]. Although many have been treated by conventional pharmaceutical approaches, there is a growing interest in the use of natural products by the general public. Hundreds of plants worldwide are used in traditional medicine as treatments for bacterial infection [2]. The interest of scientist in medicinal plants had increased rapidly due to the increased efficiency of new plant-derived drugs, growing interest in natural plants and rising concerns about the side effects of conventional medicine [3]. Several authors [4,5] defined medicinal plants as a plant in which one or more of the organs contains substances that can be used for therapeutic purposes or which it precursors for the manufacturing of drugs, are useful for disease therapy. Many of these indigenous medicinal plants are used as spices and food plants. The active components in these medicinal plants are expected to be inimical to the growth of some disease-causing microorganisms. The parts that are usually used include the leaves, fruit, seed, latex, and root. Its activity is concentration-dependent and the agent may be only static at low levels. Flavonoids, phenolic compounds, tannins and alkaloids are the most important antimicrobial agents and bioactive constituents in plants [3].

Fermentation is a process whereby microbial (yeasts and bacteria) enzymes breakdown sugars (glycolysis) and other substrates in the absence of oxygen [6]. It could be either alcoholic or acidic fermentation, which will result in the release of energy and carbon (IV) oxide. *Carica papaya* is the sole species in the genus *Carica* of the plant family *Caricaceae* [7]. It is known to botanists as *Asimina triloba*. It is called pawpaw in the English language and the Yoruba speaking tribe call it ibepe. The fruits are low in calories and rich in natural vitamins and minerals. Papaya places the highest among fruits for vitamin C, vitamin A, riboflavin, folate, calcium, thiamine, iron, niacin, potassium and fibre [8]. The chemical constituents of *Carica papaya* are Proteolytic enzymes, including papain, chymopapain, glycyl endopeptidase and carpaine; Vitamin C and beta-carotene; Lycopene [9]. Unripe, green *C. papaya* fruit seems to contain the most beneficial phytochemicals. *C. papaya* has been used as treatment for numerous maladies, ranging from gastrointestinal disorders, asthma, sexually transmitted diseases, antihelmintic, stomach ulcers, internal parasites, dysentery, malaria, jaundice, hepatitis, wounds, and burns [10]. Antimicrobial activity of Papaya may exert a Proteolytic effect on bacteria resulting from the production of a coagulum that immobilizes microorganisms and protects the host against bacterial infections [7]. In addition, papaya may improve the efficiency of phagocytic cells that destroy bacteria; Papaya also contains the alkaloid, carpaine, which has antibacterial properties [11].

2. MATERIALS AND METHODS

2.1 Sterilization of Materials

The glassware used for this research include Petri dishes, test tubes, conical flasks, beakers and MacCartney bottles. All glassware was washed with detergent and rinsed with distilled water properly. These were then air-dried before wrapping with aluminium foil and sterilized in the autoclave at 125°C. The culture media used for this research work are Nutrient agar, Eosin Methylene Blue (EMB) agar, MacConkey agar, Mueller Hinton agar. Each of the culture media was prepared and sterilized according to the

manufacturer's specification. Cork borer, glass rods, inoculating loop and forceps were sterilized by heating in Bunsen flame to redness before and after each use.

Table 1. Qualitative phytochemical components of unripe *Carica papaya*

Plant parts	phenols	Alkaloids	flavonoids	Tannins	Saponins	Glycosides
Pulp	+ve	-ve	+ve	+ve	+ve	-ve
Peel	+ve	+ve	+ve	+ve	+ve	-ve
Seed	+ve	+ve	+ve	+ve	-ve	-ve
Leaves	-ve	-ve	+ve	-ve	-ve	+ve

Key: + Positive, - = Negative

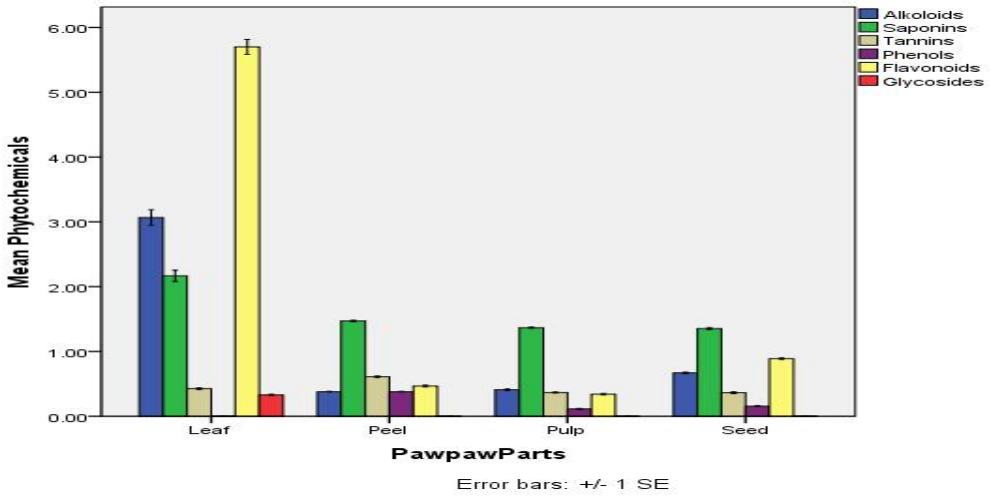

Fig. 1. Quantitative phytochemical components of *Carica papaya*

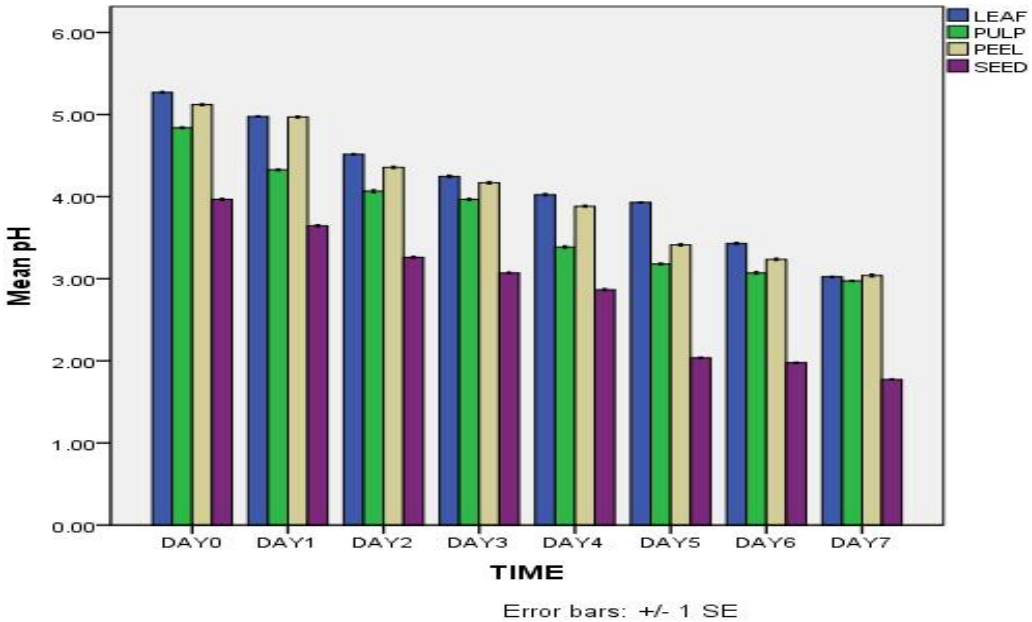

Fig. 2. The pH of the fermenting pawpaw parts against time (days)

Table 2. Proximate compositions of the fermenting unripe *Carica papaya* parts

Proximate compounds	Pulp	Peel	Seeds	leaves
Moisture Content (%)	81.39	54.48	5.91	82.00
Dry matter (%)	18.61	45.52	94.09	0.00
Crude fibre (%)	11.62	14.52	5.32	12.38
Ash (%)	4.84	5.25	7.35	1.92
Crude protein (%)	1.46	10.56	14.41	9.05
Fat (%)	0.55	0.23	5.10	3.15
Carbohydrates (%)	18.47	30.35	32.18	73.50

2.2 Collection and Maintenance of Test Organisms

The test organisms that were used were all human pathogenic organisms from clinical origin. These isolates include *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, and Proteus mirabilis.* They were obtained from the State Specialist Hospital, Akure. The organisms were collected on sterile agar slants and incubated at 37°C for 48 hours. They were then kept as stock cultures in the refrigerator set at 4°C. Biochemical analysis was carried out on each of the test organisms for confirmatory purposes.

2.3 Collection of Plant Materials

The plant *Carica papaya* (pawpaw leaf, pulp, peel and seeds) used for this project work was collected from the forest and wildlife reserve of the Federal University of Technology, Akure, Ondo State where they were found growing naturally. They were collected in a sterile polythene bag, rinsed, peeled and cut into small sizes and separated into the leaves, seeds, peel, and pulp.

2.4 Preparation of Plant Material

Unripe pawpaw leaf, pulp, seed and peel were weighed and soaked separately in a fermenting jar for eight days. This process was also repeated by soaking the leaf, pulp, seed and peel together in a jar (equal amount of each was used), and the pulp and peel were soaked together also in a fermenting jar for eight days. Three small sized unripe pawpaws were washed and peeled. The peels were kept in a different container; fresh leaves were also plucked and properly rinsed, and cut into small sizes. The weight of the peel, pulp leaf and seed soaked was 1000 g, 1000 g, 346 g and 287 g respectively. Distilled water was added to the samples to fill to saturation.

2.5 Standardization of the Test Organisms

A loop full of test organism was aseptically inoculated into 20 ml nutrient broth and incubated for 24 h. Exactly 0.2 ml from the 24 h culture of the organisms was dispensed into 20 ml sterile nutrient broth and incubated for 3- 5 h to standardize the culture to 0.5 McFarland standards (106 cfu/ml) before use according to the method of [12].

2.6 Phytochemical Analysis

Phytochemical analysis for the major components of the fermenting plant extract was carried out using standard qualitative and quantitative methods that were described by various authors [13]. The plant extract was screened for the presence of biologically active compounds like saponins, tannins, alkaloids, phenols, flavonoids, glycosides, and anthraquinones.

2.7 Proximate Analysis

The proximate analysis of the fermenting pawpaw extracts was done to divide it into six fractions which are: Moisture, ash, crude fibre, nitrogen-free extractives, crude protein, and ether extract using various standard methods.

2.8 pH

This is the hydrogen ion concentration, expressed as a negative logarithm OR measure of acidity or alkalinity of a given solution. The pH meter was plugged, switched on, and allowed to warm up for 15 minutes. It was then standardized using salts of pH 4, 7 and 9. The tip was wiped clean after dipping in each salt. It was then dipped into the fermenting samples, one after the other and the readings were taken.

2.9 Titratable Acidity

Exactly 5 mls of the samples were titrated against NaOH. Two drops of phenolphthalein

were added to the samples each in a beaker, NaOH was run into the beaker until a change in colour was observed, the colour change was from colourless or green depending on the sample to pink. The total volume of base used was recorded, and the titre value determined. This was repeated daily for each of the samples for eight days.

2.10 Microbial Load

The microbial load was done using nutrient agar and potato dextrose agar which were prepared according to the manufacturer's instructions and Serial dilutions were done to the power of 4, 6, and 9 before pouring the liquid samples into the sterile Petri dishes. The plates were seeded aseptically with 1 ml each of the fermenting liquid for each fermenting pawpaw part, and 20 ml of sterilized potato dextrose agar for fungi and nutrient agar for bacteria was poured aseptically on the seeded plates. The plates were swirled carefully for even distribution and allowed to gel. The plates were incubated at 25°C for fungi and 37°C for bacteria for 24 hours. The number of colony growth on the plates were counted and recorded.

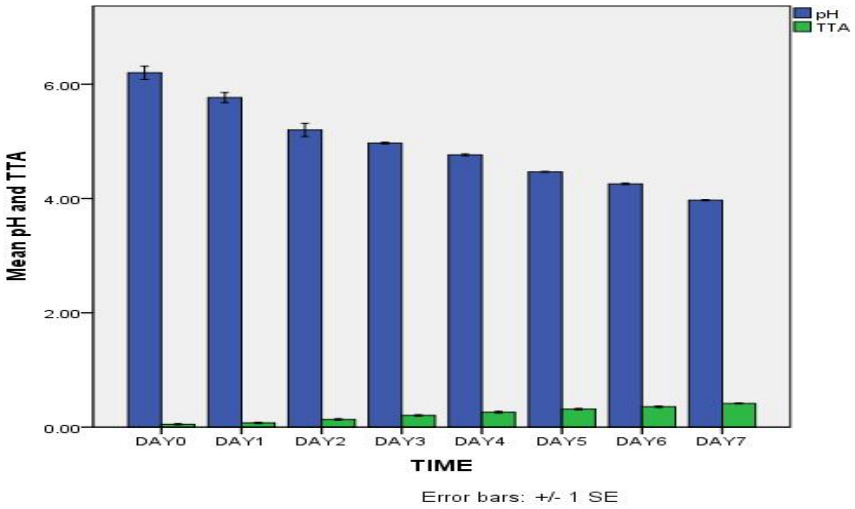

Fig. 3. The pH and titratable acidity against time for the synergism of the fermenting *Carica papaya* parts

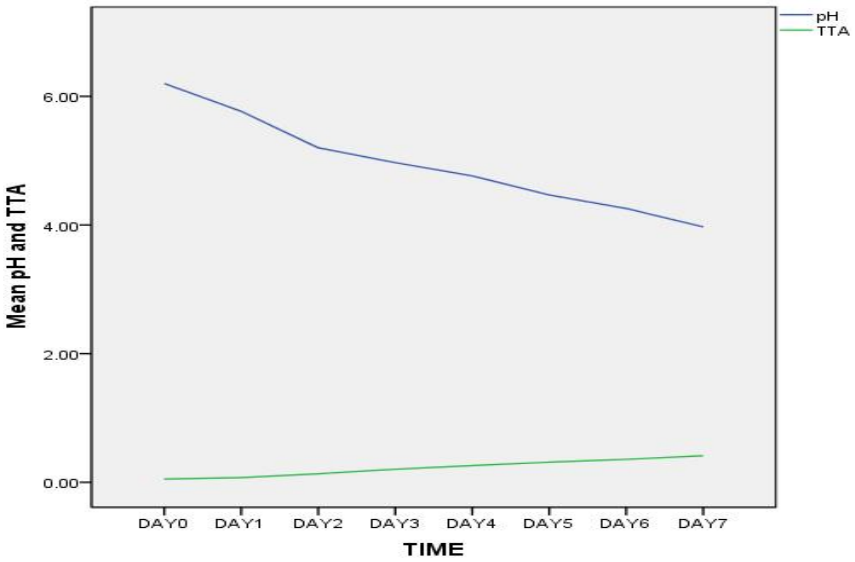

Fig. 4. pH and titratable acidity against time

2.11 Antibacterial Assay of the Fermenting Pawpaw Parts (Leaves, Seeds, Pulp and Peel)

Antibacterial activities of the extracts were determined by the agar well diffusion method as described by several authors [14,15]. The bacterial isolates were standardized to 0.5 McFarland standards (106 cfu/ml) as described by [12]. Exactly 20 mls of sterilized Mueller Hinton agar was aseptically poured into sterile Petri dishes and allowed to gel, a sterile swab stick was dipped into the standardized broth culture and streaked on the plates. This was allowed to stand for ten minutes. With the aid of sterile cork borer, four wells were borne on the solidified agar medium. Exactly 1 ml of the fermenting liquid samples were then introduced into three wells and appropriately labelled while water was introduced into the fourth well as a control. The plates were allowed to stand on the laboratory bench for 15 minutes to allow diffusion of the solution into the medium before incubating in an incubator at 37°C for 24 hours. The plates were examined for clear zones of inhibition around wells. These were measured in millimetres and the experiment was carried out in triplicates.

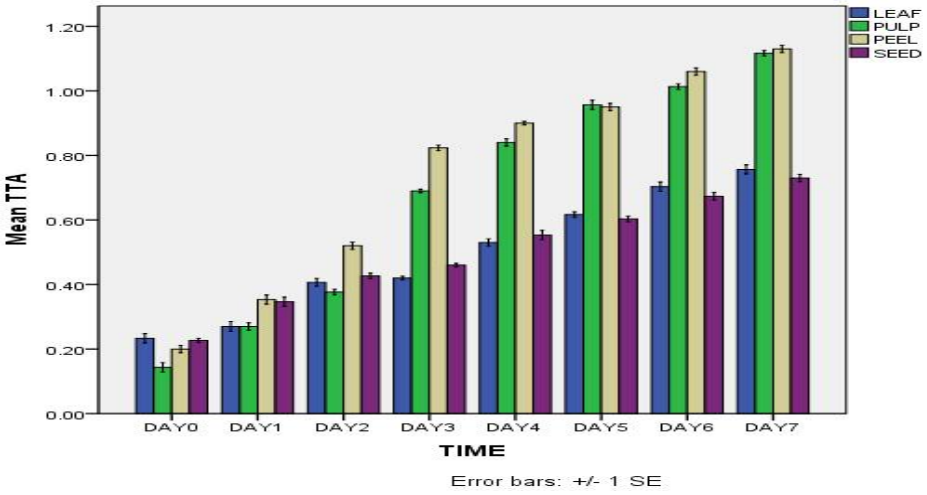

Fig. 5. Titratable acidity of the fermenting pawpaw parts against time

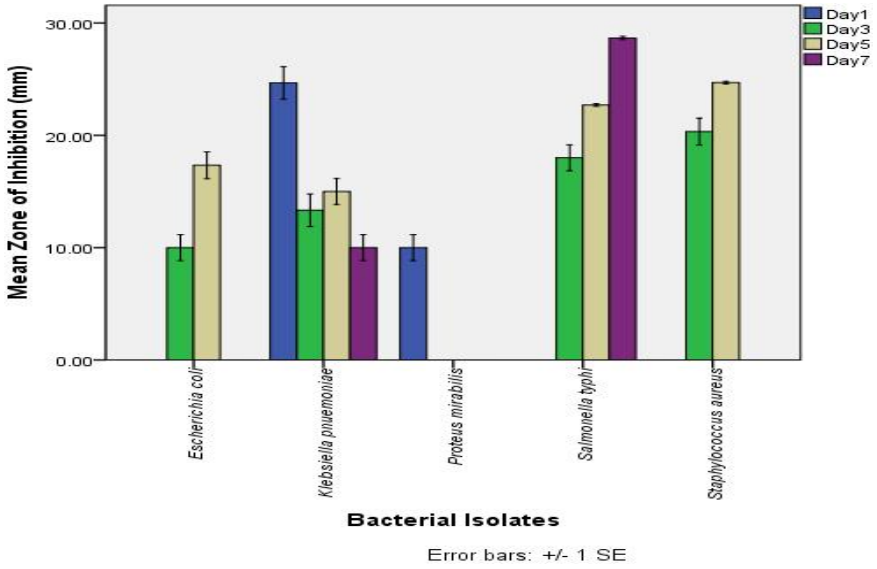

Fig. 6. Antibacterial activity of the fermenting leaf

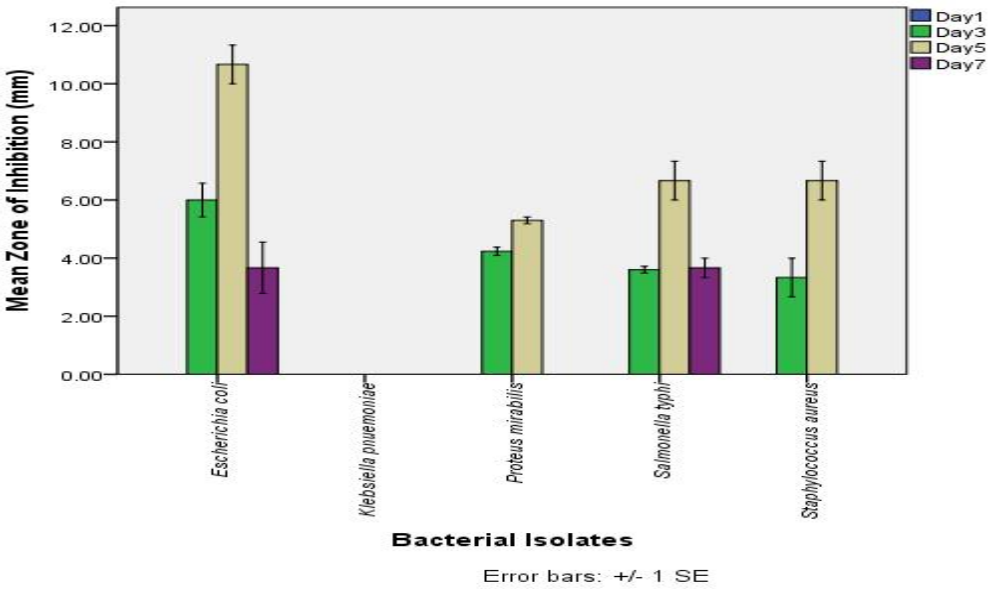

Fig. 7. Antibacterial activity of the fermenting pawpaw pulp

2.12 Antibiotic Sensitivity Test

The Kirby - Bauer test, also known as disc diffusion method described by [1] was used to determine the effect of standard antibiotics on the bacterial isolates. Sterile Petri dishes were seeded aseptically with 1 ml each of the standardized broth cultures of the test organisms while about 20 ml of sterilized Mueller Hinton agar was poured aseptically on the seeded plates. The plates were swirled carefully for even distribution and allowed to gel. With the aid of sterile forceps, the antibiotic discs were placed firmly on solidified plates and incubated for 24 hours at 37°C. After incubation, clear areas around the discs were measured, which represents the zones of inhibition [16]. Seeded agar plate without antibiotics served as the control experiment. The zones of inhibition were measured in millimetre (mm). The experiment was carried out in triplicate.

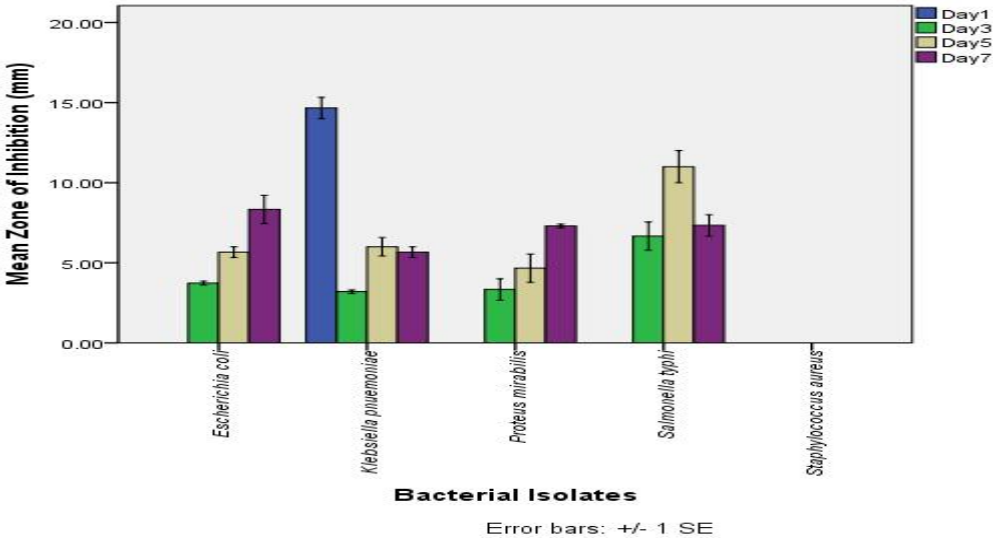

Fig. 8. Antibacterial activity of the fermenting pawpaw peel

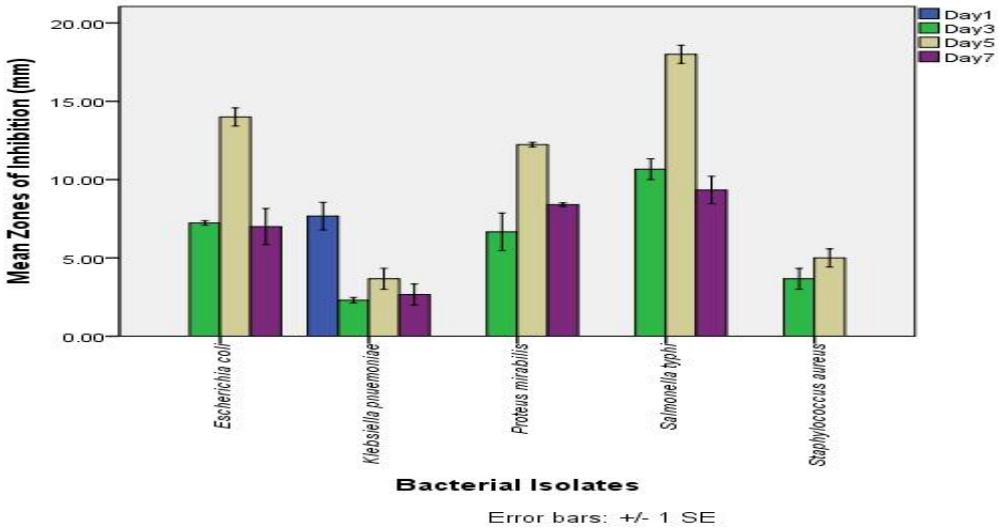

Fig. 9. Antibacterial activity for the fermentation of the pulp and peel together

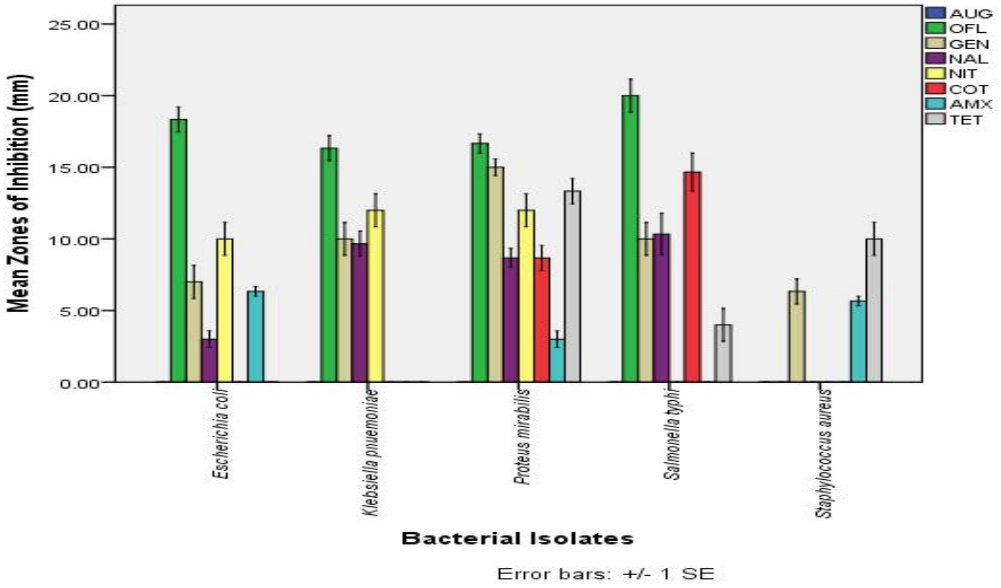

Fig. 10. Antibiotics sensitivity testing of the bacterial isolates

3. RESULTS

The test organisms that were used for this research are *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis,* and *Salmonella typhi*. The qualitative analysis of the fermenting seed, pulp and peel extracts showed the presence of tannins, saponins, phenols flavonoids, alkaloids and glycosides. The quantitative analysis revealed that the highest amount of alkaloids, saponins, and flavonoids was found in the leaf extracts while phenols were absent. Glycosides were only present in the leaf and tannins were highest in the peel extracts. The proximate analysis shows that dry matter was absent in leaf extract but highest in seeds. Moisture content, crude fibre, ash, crude protein, fat and carbohydrates were all present in the pulp, peel, seeds and leave extract. The level of acidity increased with an increase in the numbers of fermenting days. The pH decreases from about 5.29 to 1.77. The titratable acidity of the fermenting pawpaw parts increases with time from 0.143 to 1.130. The leaves, pulp and peel show inhibition against the test organisms. The highest antibacterial activity

was produced by unripe pawpaw leaf on day 7 as 28.67, while the lowest activity was observed on day 3 by unripe pawpaw pulp as 3.20. The synergism of all the pawpaw part has no antibacterial activity, while that of the peel and pulp has the highest antibacterial activity of 18.00 against *salmonella typhi* and the lowest value of 0.00 on day 1 for *Salmonella typhi*, *Proteus mirabilis, Escherichia coli* and *Staphylococcus aureus* on day 1 and 7 respectively. The antibiotics sensitivity test of the bacterial isolates shows that the highest inhibitory zones were observed in Ofloxacin (OFL) against *Salmonella typhi* (20.00), while the lowest activity was observed in Augmentin (AUG) for all the bacteria isolates (0.00).

4. DISCUSSION

The result of antibacterial susceptibility assay, phytochemical and proximate analyses of the unripe pawpaw peel, pulp, leaves and seed, before and after fermentation showed promising evidence for the antimicrobial activity of unripe Papaya parts against enteric pathogens. The phytochemical analysis revealed the presence of alkaloids, saponins, tannins, phenols, Flavonoids and glycosides in the leaves, pulp, peel and seed of the *Carica papaya* extracts. Glycosides are not present in the seed, peel and the pulp, while the leaves lack phenols. The microbial load increases from day one till day seven with diluting factors of 10 -4 to 10-9.

The pH of the fermenting pawpaw samples decreases while the titratable acidity increases. The decrease in pH will increase the acidity of the samples and acidic mediums are known to inhibit microbial growth. This might be responsible for the increase in the degree of zones of inhibitions on day five and day seven. The leaf, pulp and the peel have the highest zones of inhibition. The leaf shows the highest antibacterial activity on the seventh day on *Salmonella typhi* (28 mm) and the lowest inhibition was on day one. The pulp has a high inhibition on *Salmonella typhi* and *Klebsiella pneumonia* on day five between 8 mm and 10 mm respectively. The leaf also shows high inhibitions against *Staphylococcus aureus,* while seeds have no zones of inhibition; this shows that it does not inhibit the growth of the test organisms used. Among the Gram-positive and Gram-negative bacteria tested, the gram-negative bacteria were more susceptible to the fermented liquid from the pawpaw parts. This result, however, is a disparity with an earlier report indicating that plant extracts are more active against Gram-positive bacteria than gram-negative bacteria [17]. There may be several factors that will predispose bacteria to antibacterial agents such as previous encounters with the agent or the nature of medium used, which may affect the diffusability of the agent.

5. CONCLUSION AND RECOMMENDATION

This project work suggested that Papaya have great potential as antimicrobial agents against selected enteric pathogens and they can be used as alternative medicine in the treatment or control of enteric bacterial infections. This further supports the use of Papaya not only as food or fruits but also as an agent to prevent or control the enteric bacterial infections and also the traditional application of the plant and suggests that the plant extracts possess compounds with antibacterial properties that may be used as antibacterial agents in novel drugs for the treatment of gastroenteritis and typhoid fever. Therefore, the unripe *Carica papaya* peel, pulp, leaves and seed could be seen as a good source of useful drugs. The result of the antimicrobial sensitivity testing against both Gram-negative and Gram-positive bacteria is an indication that the plant is a potential source for production of drugs with a broad-spectrum activity. This observation is very significant because of the possibility of developing therapeutic substances that will be active against multidrug-resistant organisms.

The results of the work also support The present study suggested that the fermented unripe Papaya leaves, peel, pulp, and seeds have great potential as antimicrobial agents against selected enteric pathogens and they can be used as alternative medicine in the treatment or control of enteric bacterial infections. Phytochemical revealed the presence of medicinally important constituents in these fruit juices. Many pieces of evidence gathered in earlier studies had confirmed the identified phytochemicals to be bioactive.

The pulp leaves and the peel can be explored further for the extraction of antimicrobials by the more sophisticated procedure. Fermenting unripe pawpaw parts produced inhibitory zones that are higher than those of the antibiotics sensitivity testing. Therefore, it can be purified and further work can be done to determine the toxicological effect of the fermenting pawpaw parts on the vital

organs of the body. Further work also includes pharmacological investigation of the fermenting liquid extracts and also the investigation of Phytochemical responsible for antimicrobial activity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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