ANTIBACTERIAL PROPERTIES OF *CARICAPAPAYA* (PAWPAW) LEAF EXTRACT ON SOME PATHOGENIC BACTERIA OF PUBLIC HEALTH IMPORTANCE

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Abstract

This study investigated the antibacterial activity of *Carica papaya* leaf extracts against pathogenic bacteria, aiming to explore the potential of natural remedies in combating bacterial infections. The rising global concern of antibiotic resistance necessitates the exploration of alternative therapeutic options. *Carica papaya* leaves, known for their traditional medicinal use contain bioactive compounds with potential antimicrobial properties. The study employed in vitro assays to assess the antibacterial effects of *Carica papaya* leaf extracts against a range of pathogenic bacteria, both Gram-positive and Gram-negative. The assays revealed substantial zones of inhibition, indicating the ability of the extracts to impede bacterial growth. Minimum inhibitory concentration (MIC) values further underscored the potency of the extracts. These findings align with earlier studies identifying alkaloids, flavonoids, and phenolic compounds in *Carica papaya* leaves with antibacterial attributes. However, challenges such as variability in bioactive compound composition and lack of in vivo data warrant further research. Mechanistic studies, clinical trials, formulation development, and safety assessments are recommended to advance the understanding and potential application of *Carica papaya* leaf extracts as an alternative antibacterial treatment. Integrating traditional knowledge with modern scientific methodologies holds the promise of addressing the pressing issue of antibiotic resistance.

Key words: Antibacterial, Leaf extract, *Carica papaya*, Pathogenic bacteria, Sagbama, Bayelsa State

INTRODUCTION

In recent years, the emergence and spread of antibiotic-resistant bacteria have become a significant global health concern. Traditional antibiotics are becoming less effective in treating bacterial infections, leading to an urgent need for alternative strategies to combat pathogenic bacteria.The use of medicinal plants has been welcomed in several countries as an alternative to synthetic drugs due to their innate antimicrobial properties (Epidi *et al.,* 2016). According to World Health Organization, close to 80% of the world population utilize medicinal plants to treat human diseases (Ayoola*et al.,* 2010) The main source of antimicrobial agents has been plants in recent times (Karpagam and Nagalakshmi, 2014). Natural products have gained considerable attention as potential sources of novel antimicrobial agents due to their diverse chemical composition and complex biological activities.

One such natural product is pawpaw (*Carica papaya*), a tropical fruit wi2dely known for its nutritional and medicinal properties (Silva *et al.,* 2020). *Carica papaya* leaves have long been used in traditional medicine for their potential antibacterial properties. They contain various bioactive compounds, including alkaloids, flavonoids, and phenolic compounds, which have been reported to possess antimicrobial activities against a wide range of pathogenic bacteria (Silva, *et al.,* 2020). The *Carica papaya* plant is a nutritionally abundant source of vitamins A, B and C and also a fair source of calcium and iron (Orhue and Momoh, 2013). It contains enzyme papain, which helps in digestion and used to treat ulcers and in some microbial diseases where it is specifically effective against gram-negative bacteria at higher doses (Bibitha*et al.,* 2002).

MATERIALS AND METHODS

Sample procurement

Triplicate samples of the *Carica papaya* used in this study were obtained from Sagbama Town in Sagbama Local Government Area of Bayelsa State, Nigeria.

Sample preparation

Triplicate leave samples of *Carica papaya* were dried at room temperature. Thereafter, they were macerated using sterile pestle and mortar. The samples were further blended using electric blender to obtain fine power.

Sterilization of bench top and equipment

Materials/ equipment such as beakers, McCartney bottles, test tubes, filter papers, spatula, forceps etc used in this study were sterilized using hot air oven. The working benches were disinfected with 96% ethanol during and after working.

Extraction method

The extraction was carried out using soaking method previously described by Doherty et al. (2010) and Chiejina and Ukeh (2012), Kigigha*et al.*, (2015), Kigigha*et al.,* (2018a,b), Izah et al. (2019a,b), Izah*et al.,* (2018a,b) with slight modifications. Water, methanol and ethanol water were used for the extraction. 5g of the blended samples were extracted using 10ml of the ethanol, aqueous and methanol separately. The samples were soaked for 3 days, thereafter they were filtered with muslin cloth and the extract was collected in a conical flask. The leave extracts were further filtered using what man filter paper. The resultant filtrates were concentrated.

Source and Preparation of organisms

The microorganisms used in this study were obtained from a stock culture at the Federal Medical Centre's Microbiology and Parasitology Unit in Yenagoa, Bayelsa State, Nigeria. Following the methods previously described by Cheesbrough (2004), Benson (2002), before the sensitivity analysis, the purity of the bacteria was checked by sub culturing. The *Staphylococcus aureus* was characterized by plating on Mannitol salt Agar which showed

yellow pigmentation. It was further, grown on Nutrient Agar; the resultant growth on the Nutrient agar was subjected to coagulase test using the guide of Cheesbrough (2004).
Similarly, the *Escherichia coli* used in the study was also streaked on MacConkey agar and Levine's eosin Methylene Blue (EMB) Agar. After 24 hours of aerobic incubation at 37º C, the presence of greenish metallic sheen with small nucleated colonies in plates containing EMB (Eosine methylene blue) agar indicated the presence of *Escherichia coli* (*Pandy et al.,* 2016; Benson, 2002), while, the growth on the MacConkey agar with pinkish red growth having a metallic sheen or reflection confirmed the presence of *E. coli*. Other confirmation tests carried out include Indole, Methyl red, catalase test using the guide provided by Benson (2002) and Cheesbrough (2004). Also, the *Klebsiella* and *Micrococcus* species used in this study was confirmed by conducting some biochemical tests on the organisms including Urease, oxidase, citrate and indole using the scheme of Benson (2002) and Cheesbrough (2004).

Antimicrobial screening of the extract

Agar diffusion method was employed for the antimicrobial testing using the scheme of Lino and Deogracious (2006), Kigigha*et al*., (2015, 2016), Epidi*et al*. (2016a,b), Doherty *et al.,* (2010) with slight modification by Agu and Thomas (2012), Kigigha*et al.,* (2015), Kigigha*et al.,* (2018a,b, c, d), Izah and Aseibai (2018), Izah*et al*., (2019a,b), Izah*et al.,* (2018a,b) was employed for the sensitive assessment of the aqueous, *methanol* and ethanolic extract of *Carica papaya.* About 20ml of the autoclaved nutrient agar was poured on sterile Petri dish and allowed to solidify. Approximately 0.4ml of the test organisms was place on the agar plates and was spread over the surface using spreader. Sterilized cork borer was used to make holes approximately 6mm in the agar plates, three wells per plate (each being for the different solvents used in the study). The plates were placed inverted and labeled showing the different concentrations placed in each of the wells. About 200µl (0.2 ml) of the extract was dispensed into the agar wells made. The plates were masked with tape to avoiding shifting (disarrangement of the varying concentrations). Positive controls were established i.e. known antibiotics (1% ampiclox). All the plates were incubated at 37ºC for 24 hours under aerobic conditions. The resultant zones of inhibition were recorded in triplicates.

Statistical analysis

Statistical Package for Social Sciences (SPSS) software version 25 was used to carry out the statistical analysis. The data were expressed as Mean ± standard deviation. A one-way analysis of variance was carried out at P = 0.05, and Tukey Honestly Significant Difference (HSD) Test was used form multiple comparison between means of each of the organisms as well as based on solvents.

RESULTS

Table 1 shows the zone of inhibition exhibited by aqueous, acetone and ethanolic extracts of *Carica papaya* leaf. The zone of inhibition for *Escherichia coli, Pseudomonas species*, *Staphylococcus aureus* and *Proteus species* was 8.00mm, 8.00mm, 8.67mm and 9.00mm, respectively for aqueous extracts, 9.33mm, 9.00mm, 11.67mm and 10.00mm, respectively for acetone extracts, and 13.00, 11.33mm, 13.33mm and 12.00mm, respectively for ethanolic

extracts. There was no statistical variation (P > 0.05) in the zone of inhibition across the various isolates for each of the extracts.

Figure 1 shows the zone of inhibition exhibited by *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* and *Proteus* species when exposed to aqueous, acetone and ethanolic extracts of *Carica papaya* and Ampiclox. The zone of inhibition of test isolates when exposed to aqueous, acetone and ethanolic extracts of *Carica papaya* and Ampiclox were 8.00mm, 9.33mm, 13.00mm and 24.67mm, respectively for *Escherichia coli*, 8.67mm, 11.67mm, 13.33mm and 23.33mm respectively for *Staphylococcus aureus*, 8.00mm, 9.00mm, 11.33mm and 23.67mm respectively for *Pseudomonas* species, and 9.00mm, 10.00mm, 12.00mm and 21.00mm, respectively for *Proteus species*. Statistically, there was a variation (P < 0.05) in the solvents and Ampiclox comparison for each of the test isolates. Furthermore, Tukey Honestly Significance difference test statistics showed that mean value of Ampiclox were the predominant source of the variation observed.

DISCUSSION

Based on Table 1 and Figure 1, *Carica papaya* extracts has antibacterial activities. This is in consonance with previous works. Ebana, *et al.,*(2016) reported that petroleum ether, aqueous and ethanolic extracts of *Carica papaya* is potent against *Escherichia* coli, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Achi (2006) also reported that cold water and ethanol extracts of *Carica papaya* are potent against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Though, the zone of inhibition exhibited by the test organisms used for this study is different from the ones previously reported by authors. The variation could be due to the differences in concentration of the extracts, solvents used for extracts, physical condition i.e. age of the plant material, strain of the microbial isolates among other factors (Chiejina and Ukeh, 2012).

Authors have variously reported that the medicinal potentials of plants including Caricar papaya is due to the presence of bioactive components (Agu and Thomas, 2012 ; Benson, 2002). Several phytochemicals are found in *Carica papaya*. Achi (2006) reported the presence of tannins and glycoside and absence of include alkaloids, saponin, flavonoids and antraquinones in ethanol and cold water extracts of *Carica papaya* extract. Ebana, *et al.,*(2016) reported the presence of reducing compounds (polyphenols, phlobatannins, anthraquinones and (hydroxymethyl) anthraquinones, alkaloids, flavonoids, glycoside).

Isolates	Water	*Methanol*	Ethanol
E.coli	10.67±0.58a	11.33±1.15a	12.00±1.00a
Micrococcus species	9.33±0.58a	10.33±0.58a	11.33±0.58a
Staphylococcus aureus	11.00±1.00a	12.00±1.73a	11.67±0.58a
*Proetus*species	10.67±1.15a	12.67±0.58a	11.00±1.00a

Table 1: Zones of Inhibition (mm) of hot water, *methanol* and ethanol water leave extracts of *Carica papaya*

NOTE: Data is expressed as mean ±Standard *Deviation*; the same letters represent significant difference (P<0.05) according to Tukey Honestly Significant Difference statistics

Leaf extracts	*E.coli*	*Micrococcus* species	*Staphylococcus aureus*	*Klebsiella* species
Hot water	10.67±0.58a	9.33±0.58a	11.00±1.00a	10.67±1.15a
Methanol	11.33±1.15a	10.33±0.58a	12.00±1.73a	12.67±0.58a
Ethanol	12.00±1.00a	11.33±0.58a	11.67±0.58a	11.00±1.00a
Ampiclox	24.67±2.08b	23.67±4.04b	23.67±3.51b	23.00±3.61b

Table 2: Zones of Inhibition (mm) exhibited by *E.coli, Staphylococcus aureus, Micrococcus* and *Klebsiella* species when exposed to hot water, *methanol* and ethanolic leave extracts of *Psidiumguajava*

NOTE: Data is expressed as mean ±Standard *Deviation*; the same letters represent significant difference (P<0.05) according to Tukey Honestly Significant Difference statistics

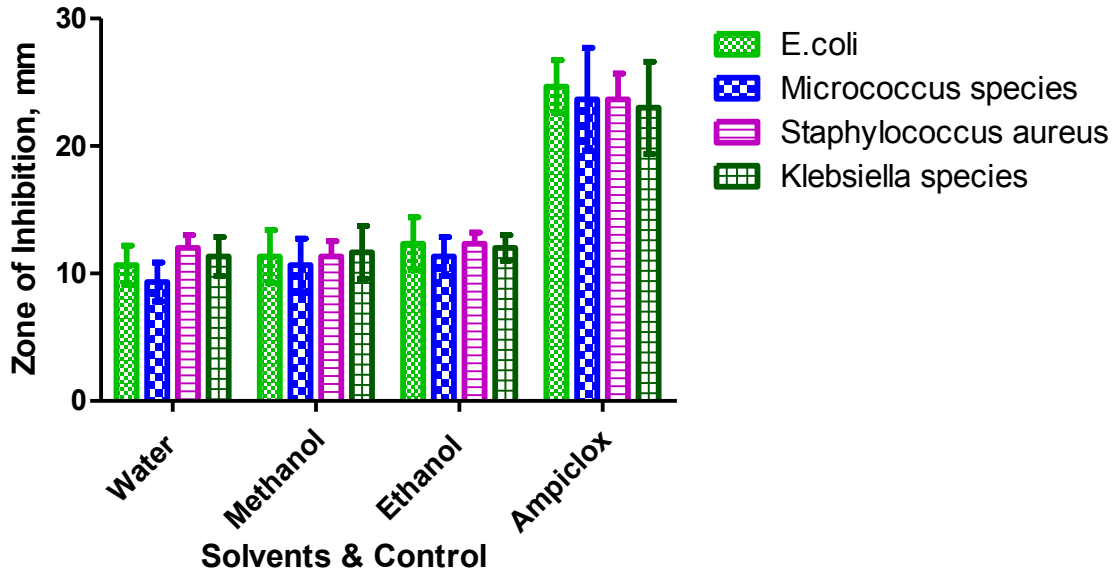

Figure 1 : Zones of Inhibition (mm) exhibited by *E.coli, Staphylococcus aureus*, *Micrococcus* and *Klebsiella* species when exposed to hot water, *methanol* and ethanolic leave extracts of *Carica papaya*

CONCLUSION

In conclusion, the investigation into the antibacterial activity of papaya leaf extracts against pathogenic bacteria has provided valuable insights into the potential of this natural remedy as an alternative or complementary therapeutic agent against bacterial infections. The findings of this study contribute to the growing body of research on the antimicrobial properties of medicinal plants and reaffirm the significance of traditional knowledge in modern scientific applications.

The results obtained from the experiments indicate that papaya leaf extracts possess substantial antibacterial effects against a range of pathogenic bacteria. The zones of inhibition observed in the assays suggest that the extracts have the ability to impede bacterial growth, and the minimum inhibitory concentration (MIC) values further emphasize the potency of the extracts in halting bacterial proliferation. These outcomes align with earlier studies that have identified

bioactive compounds in papaya leaves, such as alkaloids, flavonoids, and phenolic compounds, which have demonstrated antibacterial properties.

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