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Antibacterial Potential of Ethanolic and Aqueous Extracts of *Carica papaya* Leaves

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Authors' contributions

This work was carried out in collaboration among all authors. Author AIA conceptualized and designed the study and also wrote the manuscript. Author IUA managed the analyses of the study and the literature searches. Author AUM wrote the protocol while author JAE performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Background: The search for newer sources of antibiotics is a global challenge pre-occupying research institutions, pharmaceutical companies and academia, since many infectious agents are becoming resistant to synthetic drugs.
Aim: This present study sought to investigate the antibacterial potential of ethanolic and aqueous extracts of *Carica papaya* leaves.
Materials and Methods: Fresh and healthy leaves of *C. papaya* were harvested, air dried and milled into powder. The powder was extracted using ethanol and water as solvents. The antibacterial activities of both extracts were determined by diffusion method. Nutrient agar medium was prepared using standard method. Pure cultures of *Coliform bacillus*, *Staphylococcus epidermidis*, *Streptococcus viridans*, *Salmonella typhi* and *Escherichia coli* were obtained from the Department of Veterinary Microbiology and Parasitology, Federal University of Agriculture,

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Abeokuta, Nigeria. The extracts were serially diluted to obtain 1.0%, 0.5%, 0.25% and 0.125% solutions in sterile test tubes. Sterilized 9 mm filter paper disc soaked in the diluted extracts were placed on the plate and incubated for 24 hours at room temperature. The plates were examined for clear zones of inhibition. Presence of zones of inhibition indicated activity.
Results: the results showed that both ethanolic and aqueous extracts of *C. papaya* leaves exhibit antibacterial activities against *C. bacillus, S. epidemidis, S. viridans* and *E. coli* and also inhibited their growth. The effect of the ethanolic extract was greater than that of the aqueous extract. However, this activity was not observed with *S. typhi*.
Conclusion: The result of the present study showed that *C. papaya* leaves might effectively inhibit the growth of *C. bacillus, S. epidemidis, S. viridans* and *E. coli* but not that of *S. typhi*. However, the ethanolic extract is more potent than the aqueous extract.

Keywords: Carica papaya; C. bacillus; E. coli; S. epidemidis; S. typhi; S. viridians.

1. INTRODUCTION

Emergence of resistant strains of pathogenic microorganism has continued to pose a major health concern about the potency and efficacy of several drugs, most importantly antibiotics currently in use [1]. Thus, attention has been shifted to medicinal plants. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments [2]. In the last few years, a number of studies have been conducted in different countries to prove such efficiency [3]. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. The local use of natural plants as primary health remedies, due to their pharmacological properties, is quite common in Asia, Latin America, and Africa [4].

Carica papaya belongs to the family of Caricaceae, and several species of Caricaceae have been used as remedy against a variety of diseases [5,6]. Originally derived from the southern part of Mexico, *C. papaya* is a perennial plant, and it is presently distributed over the whole tropical area. In particular**,** *C. papaya* fruit circulates widely, and it is accepted as food or as a quasi-drug. Many scientific investigations have been conducted to evaluate the biological activities of various parts of *C. papaya*, including fruits, shoots, leaves, rinds, seeds, roots or latex. The leaves of *C. papaya* have been shown to contain many active components that can increase the total antioxidant power in blood and reduce lipid peroxidation level, such as papain, chymopapain, cystatin, à-tocopherol, ascorbic acid, flavonoids, cyanogenic glucosides and glucosinolates [7].

Fig. 1. *Carica papaya* plant [8]

Fruit and seed extracts have pronounced bactericidal activities [8]. Leaves have been poulticed into nervous pains, elephantoid growths and it has been smoked for asthma relief amongst tropical tribal communities. The hypoglycemic effect of ethanolic extract of papaya in alloxan-induced diabetes has been reported [8]. Moreover, *C. papaya* leaf juice is consumed for its purported anti-cancer activity by people living on the Gold Coast of Australia, with some anecdotes of successful cases being reported in various publications. *C. papaya* leaf extracts have also been used for a long time as an aboriginal remedy for various disorders, including cancer and infectious diseases. Airaodion, et al. [9] has reported that *C. papaya* leaves possesses antiplasmodial potency against *Plasmodium berghei* in infected Swiss albino mice.

C. papaya contains two important biologically active compounds viz., chymopapain and papain which are widely used for digestive disorders [10]. It showed that papaya derived papain,

caricain, chymopain, and glycerin endopeptidase can improve acidic pH conditions and pepsin degradation. This was reported to adversely affect fertility parameters in male Wistar rats [11]. Other active compounds of *C. papaya* are lipase, a hydrolase, which is tightly bonded to the water-insoluble fraction of crude papain and is thus considered as a "naturally immobilized" biocatalyst [12]. According to the folk medicine, papaya latex can cure dyspepsia and also applicable for external burns and scalds. Seeds and fruits are excellent antihelminthic and antiamoebic [13]. Dried and pulverized leaves are sold for making tea; also the leaf decoction is administered as a purgative for horses and used for the treatment of genetic-urinary system. This present study sought to investigate the antibacterial potential of ethanolic and aqueous extracts of *C. papaya* leaves.

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Plant Materials

Fresh and health leaves of *C. papaya* free from disease were harvested from the Institute of Agricultural Research and Training, Moor Plantation, Ibadan and were identified by a botanist. They were washed in running water to remove contaminants. They were air dried at room temperature in an open laboratory space for 14 days and milled into powder using an electric blender (Moulinex). The extraction was done using soxhlet apparatus and ethanol as the solvent according to the method described by Airaodion, et al. [14,15]. About 25 g of the powder was packed into the thimble of the soxhlet extractor. 250 mL of ethanol was added to a round bottom flask, which was attached to the soxhlet extractor and condenser on a heating mantle solvent was heated using the heating mantle and began to evaporate moving through the apparatus to the condenser. The condensate dripped into the reservoir housing the thimble containing the sample. Once the level of the solvent reached the siphon, it poured back into the round bottom flask and the cycle began again. The process was allowed to run for a total of 18 hours. Once the process was completed, the ethanol was evaporated in a rotary evaporator at 35°C. The aqueous extract was obtained by the method described by Taiwo [11]. About 25 g of the powder *C. papaya* leaves was soaked in 250 mL ofwater in a conical flask. The mixture was stirred, covered, and allowed to stand for 24 hours, and filtered using sterile Whatmann No.1 filter paper. The filtrate was concentrated to 20 ml on a water bath and evaporated to dryness at room temperature. The various extracts were used for the analysis of antibacterial activities and bacterial inhibition assay.

2.2 Determination of Antibacterial Activity

The antibacterial activity was determined by the diffusion method of Kirby Bauer described by Duguid, et al. and cited in Airaodion, et al. [16]. This method determines the antibacterial activity of the extracts.

2.3 Preparation of the Nutrient Medium

Nutrient agar medium was prepared according to the method described by Taiwo [11]. 2.8 g of nutrient agar was dissolved in 100 mL distilled water. The solution was sterilized in an autoclave at 121°C at 1.1N pressure for 15 min. The suspension was cooled and poured into sterile Petri-dishes to solidify. The agar depth of the medium was 4.0 mm.

2.4 Preparation Cultures and Inoculation

Pure cultures of *Coliform bacillus*, *Staphylococcus epidermidis*, *Streptococcus viridans*, *Salmonella typhi* and *Escherichia coli* obtained from the Department of Veterinary Microbiology and Parasitology, Federal University of Agriculture, Abeokuta, Nigeria were separately used to inoculate the Petri-dishes. This was done by streaking the surface of the plates in a zigzag manner until the entire surface was then covered [11].

2.5 Assay of Bacterial Inhibition Activity

The extracts were serially diluted to obtain 1.0%, 0.5%, 0.25%, and 0.125% solutions in sterile test tubes according to Taiwo [11]. Sterilized 9mm filter paper disc soaked in the diluted extracts were placed on the plate and incubated for 24 hours at room temperature.The plates were examined for clear zones of inhibition. Presence of zones of inhibition indicated activity. The zones were measured according to the method of Taiwo [11].

2.6 Statistical Analysis

Data were calculated using Microsoft Excel software 2013 version.

3. RESULTS

The results of antibacterial activity and inhibition of bacterial growth by the extracts are presented in Tables 1 and 2 respectively.

4. DISCUSSION

In recent years, the growing demand for herbal products has led to a quantum jump in volume of plant materials traded across the countries. However, the use and history of herbs dates back to the time of early man, who had the crudest tools as his implements and use stones to start his fire. They used herbs in their raw and cooked forms to keep fit. Since that time, the use of herbs has been known and accepted by all nations and has been known also as the first line of treatment available to man [16]. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbours an inexhaustible source of active ingredients invaluable in the management of many intractable diseases [17]. Furthermore, the active components of herbal remedies have the advantage of being combined with other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components [18].

In this study, both ethanolic and aqueous extracts of *C. papaya* leaves were observed to exhibit antibacterial activities against *C. bacillus, S. epidemidis, S. viridans* and *E. coli* (Table 1). This corresponds to the report of Taiwo [11] who studied the antibacterial activity of paw paw roots extracts. The result further showed that both ethanolic and aqueous extracts of *C. papaya* leaves did not exhibit antibacterial activities against *S. typhi* (Table 1). This is in contrast with the findings of Ogunjobi and Elizabeth [19].

Table 1. The antibacterial activity of *Carica papaya* leaf extracts

Test organism	Ethanolic extract	Aqueous extract
Coliform bacillus	+	+
Staphylococcus epidemidis	+	+
Streptococcus viridans	+	+
Salmonella typhi	–	–
Escherichia coli	+	+

+ means present while – means absent

Table 2. Inhibition of bacterial growth by *Carica papaya* leaf extracts

Test organism	Dilution (%)	Zone of inhibition (mm)	
		Ethanolic extract	Aqueous extract
Coliform bacillus	1.00	6.00	2.50
	0.50	4.50	1.50
	0.25	3.00	1.00
	0.125	1.50	0.00
Straphylococcus epidermidis	1.00	4.50	2.00
	0.50	2.50	1.00
	0.25	2.00	0.00
	0.125	1.50	0.00
Streptococcus viridans	1.00	7.00	3.50
	0.50	4.00	2.50
	0.25	2.50	1.00
	0.125	1.00	0.00
Salmonella typhi	1.00	0.00	0.00
	0.50	0.00	0.00
	0.25	0.00	0.00
	0.125	0.00	0.00
Escherichia coli	1.00	3.50	2.00
	0.50	2.50	1.50
	0.25	1.50	1.00
	0.125	1.00	0.00

Anibijuwon and Udeze [17] extracted bioactive compounds from leaf of *C. papaya* using water and organic solvents, which were investigated for antimicrobial activity against some pathogenic organisms of clinical origin from South-Western Nigeria. Ocloo, et al. [20] studied the efficacies of crude extracts of *C. papaya* seeds against *Staphylococcus aureus*, *Escherichia coli* and *Shigella flexneri* using disc diffusion method. The crude organic (acetone, methanol) extracts inhibited the growth of all three organisms. Mahmood, et al. [21] investigated the wound healing potential of aqueous extract of *C. papaya* leaves in rats. Their results strongly documented the beneficial effects of different parts of this plant extract for the acceleration of wound healing process in rats.

The results of the inhibition of bacterial growth by both ethanolic and aqueous extracts of *C. papaya* showed that both extracts exhibited highest antibacterial activity against Gram negative organism. This is in agreement with earlier reported studies [22]. Nirosha and Mangalanayaki [23] also reported Gram negative bacteria are more susceptible to the extracts of papaya leaf and stem. But the results of Suresh, et al. [24] showed the antibacterial activity of the papaya leaf extract was more pronounced on Gram positive than Gram negative bacteria. According to Jigna and Sumitra [25], the plant leaf-extracts are more active against Gram positive than Gram negative bacteria. Our investigation results are contrary with their findings. Ogunjobi and Elizabeth [19] reported that leaf and seed extract of *C. papaya* have inhibitory effect on *Staphylococcus aureus, Shigelladysenteria, Pseudomonas aeruginosa, Pseudomonas fluroescens,* and *Salmonella typhi*. They also demonstrated that ethanol extracts contributed much more antibacterial activity than aqueous extract [26]. This present study is in consonance with their results. The highest antibacterial activity brought about by ethanolic extract might be due to better solubility of the active components of leaves in ethanol than water. This is consistent with the findings of Airaodion, et al. [16] who investigated the antibacterial activity of ethanolic and aqueous extracts of *Vernonia amygdalina* leaves against Gram-positive and Gram-negative bacteria.

5. CONCLUSION

The result of the present study showed that *C. papaya* leaves have the potentials of inhibiting the growth of *C. bacillus, S. epidemidis, S. viridans* and *E. coli* but not that of *S. typhi*. However, the results show that ethanolic extract is more potent than the aqueous extract.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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