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Comparative studies on antimicrobial properties of extracts of fresh and dried leaves of *Carica papaya* (L) on clinical bacterial and fungal isolates

Okunola A. Alabi*[1], Muyideen T. Haruna[2], Chinedu P. Anokwuru[3], Tomisin Jegede[1], Harrison Abia[1], Victor U. Okegbe[1] and Babatunde E. Esan[1]

[1]*Department of Biosciences and Biotechnology, School of Science and Technology, Babcock University, Nigeria*
[2]*Department of Medical Laboratory Sciences, Benjamin Carson's (Snr) College of Medicine, Babcock University Nigeria*
[3]*Department of Basic Sciences, School of Science and Technology, Babcock University, Nigeria*

ABSTRACT

The efficacy of treatments with C. papaya is dependent on the quantity of the different chemical substances present in the preparation. The quantity of chemical substances varies in the fruit, latex, leaves, and roots and varies with the extraction method, age of the plant part, and the cultivar and sex of the tree. The antibacterial and antifungal ability of both fresh and dried leaves of C. papaya against bacteria and fungi of medical importance was carried out. The aqueous, ethanol and acetone extract of both the dried and fresh leaves were tested at 25, 50 and 100 mg/ml concentrations on both the bacteria and fungi isolates using the disc diffusion method. Results showed very significant broad spectrum antimicrobial activity against Gram-negative and Gram-positive bacteria and fungi. The organic extracts were more effective than aqueous extracts. The result further showed that the dry sample was effective against both Gram-positive and Gram-negative bacteria while the fresh sample was more effective against Gram-negative bacteria. The dried leaf extract was potent against some of the bacteria which standard antibiotics were not able to inhibit. C. papaya leaves showed a better antibacterial activity than antifungal activity. Demonstration of antimicrobial activity against the test isolates is an indication that there is possibility of sourcing alternative antibiotic substances in this plant for the development of newer antibacterial agents.

Keywords: *Carica papaya*, antimicrobial, antibacterial, antifungal, sensitivity, zone of inhibition.

INTRODUCTION

The frequency and diversity of life-threatening infections caused by pathogenic microorganisms has increased steadily worldwide and it is becoming an important cause of morbidity and mortality in immunocompromised patients especially in developing countries [1]. Infectious diseases are the world's major threat to human health and account for almost 50 000 deaths every day [2]. Emergence of resistant strains of pathogenic microorganism has also continued to pose a major health concern about the efficacy of several drugs, most importantly antibiotics in current use [3]. This increasing rate of development of resistance to commonly used antibiotics has led to the search for newer, more effective, affordable and readily available sources, in particular, from local medicinal plants (herbs) [4]. Plants are the most naturally effective and cheapest sources of drugs [5-7]. The use of local plants as primary health remedies, due to their pharmacological properties, is quite common in Asia, Latin America, USA, China,

Japan and Africa [8]. The plant kingdom synthesizes diverse active compounds which are valuable in the treatment and control of many diseases. These compounds are principally secondary metabolites. Some of the active compounds do occur singly or in combination with other inactive substances which inhibit greatly the life processes of microbes, especially the pathogenic microbes. Medicinal plants are cheap and renewable source of pharmacologically active substances [9].

Medicinal plants are reservoirs of various metabolites and provide unlimited source of important chemicals that have diverse biological properties and represents a rich source from which antimicrobial agents can be obtained [3].The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties [10]. Antimicrobials of plant origin effective in the treatment of infectious diseases and simultaneously mitigating many of the side effects often associated with synthetic antimicrobial agents have been discovered [11-12]. Medical uses of plants range from the administration of the roots, barks, stems, leaves and seeds to the use of extracts and decoction from the plants [13].
Carica papaya, belongs to the family of Caricaceae, and several species of Caricaceae have been used as remedy against a variety of diseases [14]. Papaya offers not only the luscious taste but is a rich source of antioxidant nutrients such as carotenes, vitamin C and flavonoids; the B vitamins, folate and pantothenic acid; and the minerals, potassium and magnesium; and fiber [15]. Together, these nutrients promote the health of the cardiovascular system and also provide protection against colon cancer. The fruit is valued for its proteolytic enzymes including papain, which is used like bromelain, a similar enzyme found in pineapple, to treat sports injuries, other causes of trauma, and allergies [16]. Biochemically, its leaves and fruit are complex, producing several proteins and alkaloids with important pharmaceutical and industrial applications [17]. *Carapine*, an alkaloid present in papaya, can be used as a heart depressant, amoebicide and diuretic. The fruit and juice are consumed for gastrointestinal ailments; a fresh leaf poultice is used to treat sores. The fresh root with sugarcane alcohol can be taken orally or as a massage to soothe rheumatism. A flower decoction is taken orally for coughs, bronchitis, asthma and chest colds. In some countries, the seeds are used as an abortifacient and vermifuge. Medical research in animals has confirmed the contraceptive and abortifacient capability of papaya. Its seeds also have contraceptive effects in adult male Langur Monkeys, possibly in adult male humans [18-20]. The seed of papaya has antimicrobial activity against *Trichomonas vaginalis* trophozoites. It could also be used in urinogenital disorder like trichomoniasis with care to avoid toxicity [21]. The seeds, irrespective of its fruit maturity stages have bacteriostatic activity on gram positive and negative organisms which could be useful in treating chronic skin ulcer. However, little information [22] exits on the antimicrobial property of *C. papaya* dried and fresh leaves. In this study, we investigated the antibacterial and antifungal ability of both fresh and dried leaves of *C. papaya* against bacteria and fungi of medical importance.

MATERIALS AND METHODS

Collection of plant samples

Healthy disease free, mature fresh plant leaf samples of *C. papaya* were collected locally from Babcock University campus, Ilisan Remo, Ogun State. The fresh leaves were rinsed thoroughly 2-3 times with running tap water and once with sterile water and grounded into fine texture using an electric blender, kept in a beaker, sealed and then placed in a cool place prior to its use for the extraction. Also some of the leaves were air-dried for 2 weeks and grounded into fine texture using an electric blender. The dried leaves were stored in sealed and labeled containers for use.

Plant extract preparation (fresh and dried leaves)

C. papaya fresh and dried leaves were separately extracted with three solvents; ethanol, acetone and water, using the method as described by Oyagade *et al*. [23].

The fresh water extract was prepared by suspending 100grams of the finely blended fresh and dried leaves in 200ml of distilled water. This was then agitated using the blender after which another 300ml of distilled water was added. The mixture was stirred every 3 minutes for 30 minutes and then allowed to stand for 24 hours. The extract was then decanted and filtered through a Whatman filter paper. The filtrate was then concentrated with the rotary evaporator at 45°C. This extract was then stored in the refrigerator at 4°C until use. The ethanol extract was prepared by suspending 100grams of the finely blended fresh and dried leaves in 500ml of 95% ethanol. The mixture was then treated as described for the aqueous extract. The acetone extract was prepared by suspending 100grams of the finely blended fresh and dried leaves in 500ml of 95% acetone and subsequently processed as the other extracts.

Preparation of different concentrations of the extracts

The stock (200mg/ml) was prepared by reconstituting 4g of each of the extracts in 20ml of their respective solvent. Different concentrations (100mg/ml, 50mg/ml and 25mg/ml) of each of the extracts were then prepared from their stock. In preparing the 100mg/ml concentrations, 5ml of the different stock solutions of the extracts were transferred to a 10ml volumetric flask and made to mark with their respective solvents. For the 50mg/ml preparation, 2.5ml of the different stock solutions of the extracts were transferred to a 10ml volumetric flask and made to mark with their respective solvents. For the 25mg/ml preparation, 1.25ml of the different stock solutions of the extracts were transferred to a 10ml volumetric flask and made to mark with their respective solvents.

Collection and maintenance of test organisms

The test organisms used are all human pathogenic organisms of clinical origin. They include five strains of Gram-positive bacteria, seven strains of Gram-negative bacteria and six fungi isolates. The Gram-negative isolates include three strains of *Escherichia coli* - *E. coli* (ATCC 23922), *E. coli* (ATCC 25922) *and E. coli* (ATCC 35218)*; Klebsiella pneumoniae* (ATCC 700603), *K. oxytocum, Pseudomonas aeruginosa* (ATCC 27853) and *Proteus vulgaris* (ATCC 13315). The Gram-positive isolates include three stains of *Staphylococcus aureus* - *S. aureus* (ATCC 29213), *S. aureus* (ATCC 55620) and *S. aureus* (ATCC 25923); *Enterococcus faecalis* (ATCC 29212) and *Streptococcus pyogenes* (ATCC 8662). The fungi isolates include: *Aspergillus niger, A. flavus, A. carbonerius, Epidermophyton floccosum, Trichophyton metagrophytes and Candida albicans*. They were obtained from the Department of Medical Laboratory Sciences, School of Medical Sciences, Babcock University, Ilisan-Remo, Ogun State. They were kept as stock cultures at 4°C. Biochemical analysis was carried out on each of the test organisms for confirmation.

Antimicrobial Assay of the Extracts

Antimicrobial susceptibility test for Bacteria

The disc diffusion method was used to determine the antibacterial activity of the plant extracts. A loop-full each of the twelve bacteria were introduced separately by streaking on petri dishes containing Mueller Hinton agar which had already set and were then labelled accordingly. The plates were cultured at 37°C for 10mins after which a sterile 5mm cork borer was used to make holes (wells) in the inoculated agar. The wells formed were filled with each concentration of the extract. This was done for each of the concentrations of each extract for both the dried and fresh leaves. These were then left on the bench for 1hour for adequate diffusion of the extracts and thereafter were incubated at 37°C for 24hours. After incubation, the diameter of the zone of inhibition around each well was measured to the nearest millimetre along two axes (i.e. 90° to each other) and the mean of the two readings was then calculated.

Antibiotic sensitive test

Antibiotic susceptibility test was carried out on the test bacteria as control. A multi-sensitivity disc bearing ten different antibiotics (OFL, TET, NIT, COT, NAL, GEN, AUG, AMX, CHL and ERY) was used against each of the test bacteria inoculated on Mueller Hinton agar plates. These were incubated at 37°C for 24hours. After incubation, the diameter of the zone of inhibition around each well was measured to the nearest millimetre along two axes (i.e. 90° to each other) and the mean of the two readings was then calculated.

Antimicrobial susceptibility test for fungi

Potato Dextrose Agar (PDA) was used for the inoculation of the studied fungi. Stock cultures were maintained at room temperature on PDA. Active cultures for experiments were prepared by seeding a loopful of fungi into Potatoes dextrose broth and incubated without agitation for 48 hrs at 25^{O}C. The broth was diluted with Potatoes dextrose broth to achieve optical densities corresponding to 2.0×10^{-5} spore/ml for the fungal strains. The disc diffusion method was also used to screen for antifungal properties. PDA plates were inoculated with 1ml of the test culture, spread and the excess drained off. The plate was incubated at room temperature for 10 minutes. A sterile 5mm cork borer was used to make ditches on each plate and filled with the different concentrations of the various extracts. The same was repeated for each fungus using the different concentrations of the various extracts. The plates were incubated at 25^{O}C for 96hrs and the resulting zone of inhibition around the ditches was observed for measurement. Control test was carried out using 10mg/ml of Fluconazole.

Determination of Minimum Inhibitory Concentration (MIC)

In determining the minimum bacterial growth inhibition of the various extracts, different increasing concentration of the extracts was utilized. 2 mls of nutrient broth was prepared into test tubes for each extract and and 0.5 ml of 5 –

150 mg/ml of each extract was added to the different test tubes containing the nutrient broth. This was prepared for each organism in duplicate. A colony of 24hrs cultured-organism was inoculated into test tube containing 1ml of normal saline to form a turbidity of 0.5 McFarland standard and was thereafter dispense into the test tube containing the suspension of nutrient broth and the different extracts. This was done for all the organisms at the tested concentrations. All test tubes were properly corked and incubated at 37^{O}C for 24hrs and 25 ^{O}C for 96hrs for the bacteria and fungi respectively. After which they were observed for absence or present of visible growth. The lowest concentration without visible growth (turbidity) of organisms was regarded as the MIC.

RESULTS AND DISCUSSION

Susceptibility of the test bacteria to the extracts

The *in vitro* susceptibility of some bacteria and fungi to different concentrations of extract from both fresh and dried leaves of *Carica papaya* was determined. Table 1 shows the result of the zone of inhibition observed in the plates of bacteria exposed to different concentrations of the extracts. In the fresh leaf extract, among the cultures treated with aqueous extract, only *S. aureus* (ATCC 25923) was inhibited; *E. coli* (ATCC 23922), *K. oxytocum* and *E. coli* (ATCC 25922) were inhibited by the ethanol extract while *S. aureus* (ATCC 55620) and *S. aureus* (ATCC 25923) were inhibited by the acetone extract. The aqueous extract against *S. aureus* (ATCC 25923) yielded the highest inhibition value while ethanol extract gave the greatest number of inhibition, i.e. more test bacteria were susceptible to ethanol extract. In the dried leaf extract, the organic extracts (ethanol and acetone) were more effective on the tested organisms. The ethanol extract was more potent, inhibiting all the isolates with the exception of *S. aureus* (ATCC 29213), *K. oxytocum* and *S. aureus* (ATCC 25923) with highest inhibition of 15 mm each shown on *E. coli* (ATCC 23922), *Enterococcus faecalis* (ATCC 29212) and *Streptococcus pyogenes* (ATCC 8662). *E. coli* (ATCC 25922), *E. coli* (ATCC 35218), *K. pneumoniae* (ATCC 700603) and *S. aureus* (ATCC 55620), however, show a lowest inhibition of 10 mm in the ethanol extract which is also the lowest in all the extracts used. Acetone extract on the other hand inhibited the growth of *K. pneumonia* (ATCC 700603), *Enterococcus faecalis* (ATCC 29212), *E. coli* (ATCC 25922) and *S. aureus* (ATCC 55620). The aqueous extract showed inhibition only on *S. aureus* (ATCC 29213) with zone of inhibition of 24 mm which is the widest among all the extracts.

Table 1: The result of the zone of inhibition observed in the plates of bacteria exposed to different concentrations of various extracts of fresh and dried leaves of *Carica papaya*.

Organisms	Extraction method (Dried extract, mg/ml; mean, mm)									Extraction method (Fresh extract, mg/ml; mean, mm)								
	Aqueous			Ethanol			Acetone			Aqueous			Ethanol			Acetone		
	25	50	100	25	50	100	25	50	100	25	50	100	25	50	100	25	50	100
E. coli (ATCC 23922)	0	0	0	0	0	15	0	0	0	0	0	0	0	0	14	0	0	0
Klebsiella pneumoniae (ATCC 700603)	0	0	0	0	0	10	0	0	14	0	0	0	0	0	0	0	0	0
Enterococcus faecalis (ATCC 29212)	0	0	0	0	0	15	0	0	13	0	0	0	0	0	0	0	0	0
S. aureus (ATCC 29213)	0	9	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptococcus pyogenes (ATCC 8662)	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0
E. coli (ATCC 23922)	0	0	0	0	0	10	0	0	15	0	0	0	0	0	0	0	0	0
S. aureus (ATCC 55620)	0	0	0	0	0	10	0	0	15	0	0	0	0	0	0	0	0	9
Pseudomonas aureginosa (ATCC 27853)	0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0
Klebsiella oxytocum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0
S. aureus (ATCC 25923)	0	0	0	0	0	0	0	0	0	0	11	28	0	0	0	0	0	9
Proteus vulgaris (ATCC 13315)	0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0
E. coli (ATCC 35218)	0	0	0	0	0	10	0	0	0	0	0	0	0	0	13	0	0	0

Susceptibility of the test fungi to the extracts

Table 2 shows the result of the zone of inhibition observed in the plates of fungi exposed to different concentrations of the extracts. Only the aqueous extract of the fresh leaves was potent against the fungi isolates used in this study. *Candida albicans*, *Aspergillus flavus* and *T. metagrophytes* were the only fungi inhibited by the aqueous extract of the fresh leaves with the plate of *A. flavus* producing the widest zone of inhibition. There was no susceptibility observed in the ethanol and acetone extracts of the fresh leaves while all the three extracts from the dried leaves showed no inhibition of all the tested fungi isolates.

Antibiotics sensitive test

Table 3 shows the susceptibility of the test organisms to the different antibiotics. All the test bacteria were inhibited by at least one antibiotic except for *S. aureus* (ATCC 29213) and *Streptococcus pyogenes* (ATCC 8662) which were

resistant to all the antibiotics. *K. pneumonia* (ATCC 700603) and *P. vulgaris* (ATCC 13315) were however sensitive to only gentamicin and tetracycline respectively.

Table 2: The result of the zone of inhibition observed in the plates of fungi exposed to different concentrations of various extracts of fresh and dried leaves of *Carica papaya*.

Organisms	Extraction method (Dried extract, mg/ml; mean, mm)									Extraction method (Fresh extract, mg/ml; mean, mm)								
	Aqueous			Ethanol			Acetone			Aqueous			Ethanol			Acetone		
	25	50	100	25	50	100	25	50	100	25	50	100	25	50	100	25	50	100
Epidermophyton floccosum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspergillus niger	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspergillus flavus	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Trichophyton metagrophytes	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Candida albicans	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Aspergillus carbonerius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3: Result of the Antibiotic Susceptibility of the Test Organisms

Organisms	Antibiotic Susceptibility
E. coli (ATCC 23922)	OFL, TET, NIT, COT, NAL
Klebsiella pneumonia (ATCC 700603)	GEN
Enterococcus faecalis (ATCC 29212)	OFL, COT, GEN
S. aureus (ATCC 29213)	No susceptibility
Streptococcus pyogenes (ATCC 8662)	No susceptibility
E. coli (ATCC 23922)	TET, OFL, NAL, GEN, NIT, COT
S. aureus (ATCC 55620)	GEN, COT, CHL, ERY
Pseudomonas aeruginosa (ATCC 27853)	OFL, GEN, NIT, TET, AMX, COT
Klebsiella oxytocum	TET, OFL, COT, NIT, GEN
S. aureus (ATCC 25923)	TET, CHL
Proteus vulgaris (ATCC 13315)	TET
E. coli (ATCC 35218)	NIT, OFL, GEN

OFL = Ofloxacin *GEN = Gentamicin*
TET = Tetracyclin *AUG = Augumentin*
NIT = Nitrofurantoin *AMX = Amoxicillin*
COT = Co-trimoxazole *CHL = Chloramphenicol*
NAL = Nalidixic acid *ERY = Erythromycin*

Table 4: Minimum Inhibitory Concentration (MIC) of different concentrations of various extracts of fresh and dried leaves of *Carica papaya*.

Organisms	Extraction method (Dried extract, mg/ml)			Extraction method (Fresh extract, mg/ml)		
	Aqueous	Ethanol	Acetone	Aqueous	Ethanol	Acetone
E. coli (ATCC 23922)	100	75	100	100	75	100
Klebsiella pneumonia (ATCC 700603)	100	75	75	100	100	100
Enterococcus faecalis (ATCC 29212)	100	75	100	100	100	100
S. aureus (ATCC 29213)	25	100	100	100	100	100
Streptococcus pyogenes (ATCC 8662)	100	100	100	100	100	100
E. coli (ATCC 23922)	100	75	100	100	100	100
S. aureus (ATCC 55620)	100	75	75	100	100	100
Pseudomonas aureginosa (ATCC 27853)	100	100	100	100	100	100
Klebsiella oxytocum	100	100	100	100	75	100
S. aureus (ATCC 25923)	25	100	100	75	100	100
Proteus vulgaris (ATCC 13315)	100	75	100	100	100	100
E. coli (ATCC 35218)	100	75	100	100	100	100
Epidermophyton floccosum	-	-	-	-	-	-
Aspergillus niger	-	-	-	-	-	-
Aspergillus flavus	-	-	-	100	-	-
Trichophyton metagrophytes	-	-	-	100	-	-
Candida albicans	-	-	-	100	-	-
Aspergillus carbonerius	-	-	-	-	-	-

-=no inhibition

Determination of Minimum Inhibitory Concentration (MIC)

Table 4 shows the results of MIC determination of the various extracts of both the fresh and dried leaves on the test organisms. The lowest MIC of 50 mg/ml was demonstrated against *S. aureus* (ATCC 29213) and *S. aureus* (ATCC 25923) for the aqueous extraction of dried and fresh leaves respectively, while the MIC values ranging between 75-100 mg/ml were demonstrated against the rest of the test bacteria. There was no inhibition at all the tested concentrations for the fungi in both fresh and dried samples except for aqueous extract of the fresh sample with a MIC of 100 mg/ml on *A. flavus, T. metagrophytes* and *C. albicans.*

Plant products, particularly extracts of various plant parts have been used extensively as natural antimicrobials and antioxidants. The presence of bioactive substances has been reported to confer resistance to plants against bacteria, fungi and pests and therefore explains the demonstration of antibacterial activity by the plant extracts used in this study [24]. Results of this study revealed very significant antimicrobial activity with the extracts demonstrating broad spectrum of activity against both bacteria (*S. pyogenes, E. c*oli, *K. pneumonia, K. oxytocin, E. faecalis, P. aeruginosa, P. vulgaris* and *S. aureus*) and fungi (*A. flavus, T. metagrophytes* and *C. albicans*). The organisms used in this study are associated with various forms of infections in humans. The bacteria are associated with infections of the upper respiratory tract (*S. pyogenes*), gastrointestinal infections, dysentery and urinary tract infections (*E. coli*), neonatal nosocomial infections (*K. pneumonia*), pulmonary tract infections (*P. aeruginosa*), focal lesions (*P. vulgaris*), and urinary tract infections (*S. aureus*). However, the fungi are associated with systemic mycosis and aflatoxin production (*A. flavus*), tinea barbae (*T. metagrophytes*) and candidiasis (*C. albicans*) [25]. The demonstration of activity against all these organisms had shown that *C. papaya* can be used to produce raw materials/substances for further development of diverse antibiotics with broad spectrum of activity.

In the bacterial test, the results of this study demonstrated that the organic extracts were more effective than aqueous extracts. This may be due to the better solubility of the active components in organic solvents [26]. The ethanol extracts demonstrated a higher activity than the acetone extracts in both the dried and fresh leaf samples. The better efficacy of the ethanol extract as against the acetone extract maybe because different solvents have different polarities, hence different degrees of solubility for the various phytoconstituents [27-28]. Based on the limited spectrum of activity of the other extracts compared with the ethanol extracts, it suggests that the active component is more soluble in ethanol than in the other solvents. The result further showed that the dried sample was effective against both Gram-positive and Gram-negative bacteria while the fresh sample was more effective against Gram-negative bacteria. The fact that the dried sample extracts were active against both gram-negative and gram-positive bacteria tested may indicate a broad spectrum of activity. This result is very significant because of the possibility of developing therapeutic substances that may be more active against multidrug-resistant organisms. This observation is in accordance with the reports of Doughari *et al*. [29] and Alo *et al*. [30]. There may be several factors that will predispose bacteria to antibacterial agents such as previous encounters with the agents or the nature of medium used, which may affect the diffusability of the agent.

The result also showed that the dried leaf extract was potent against some of the bacteria (*S. aureus* (ATCC 29213) and *Streptococcus pyogenes* (ATCC 8662)) which standard antibiotics were not able to inhibit. The disparity between the activities of the extract and the standard antimicrobial drug may be due to the mixtures of bioactive compounds present in the extract compared to the pure compound contained in the standard antibiotics [31]. The demonstration of activity against the test bacteria provides scientific bases for the local usage of these plants in the treatment of various ailments.

Furthermore, *C. papaya* leaves showed a better antibacterial activity than antifungal activity. The dry sample was not effective against any of the fungi used with only the aqueous extract of the fresh sample inhibiting three (*A. flavus, T. metagrophytes* and *C. albicans*) of the six fungi studied. The zones of inhibition of the fungi were the least of the various zones of inhibition recorded in this study. This therefore suggests that this plant part is better used for the treatment of bacteria than for the treatment of the studied fungi. The efficacy of treatments with *C. papaya* is dependent on the quantity of the different chemical substances present in the preparation. The quantity of chemical substances varies in the fruit, latex, leaves, and roots and varies with the extraction method, age of the plant part, and the cultivar and sex of the tree [32].

The MIC result showed that increasing concentration has an increasing efficiency in inhibiting the organisms used. Since the MIC values indicated the definite nature of the antimicrobial activities of this plant, the inhibition zones values, only, indicated extent of effectiveness of the extract with increasing concentration.

Although the mechanism of action of this extract is not understood. It has been proposed that its action against the bacteria and fungi may be due to the inhibition of cell wall formation in the cell resulting in a leakage of cytoplasmic constituents by the bioactive components of the extract [33-34]. While phytochemical compounds such as tannin coagulate the wall proteins, saponins facilitated the entry of toxic material or leakage of vital constituents from the cell [35]. Flavonoids inhibit the activity of enzymes [36] by forming complexes with bacterial cell walls, extracellular and soluble proteins, more lipophilic flavonoids disrupt cell wall integrity [37] or microbial membranes [38] at low concentrations.

In conclusion, plant-based antimicrobials have enormous therapeutic and preferential potential. They can serve the desired purpose with lesser side effects that are often associated with synthetic antimicrobials [11]. The antimicrobial activity of *C. papaya* leaves was demonstrated in this study. Demonstration of antibacterial activity against the test isolates is an indication that there is possibility of sourcing alternative antibiotic substances in these plants for the development of newer antibacterial agents.

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