

Antimicrobial activities of *Carica papaya* leaf against diarrhoea causing agents.

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Abstract— *The advent of science to the search for antibiotics principally depends on medicinal plants as raw materials. This present study evaluated the antimicrobial effect of Carica papaya leaf extracts against bacterial and fungal agents that causes diarrhoea. Fresh tender roots and leaves of this plant was collected, air-dried, powdered and percolated in n-hexane, methanol and aqueous solvents. The antimicrobial activities of the extract against test organisms were tested by using agar well diffusion assay and the MIC, MBC and MFC values were determined by agar dilution assay. The results revealed that the crude methanol and aqueous extracts of Carica papaya had no anti-fungal activity, but have antibacterial activity. N.hexane extract of C. papaya had most activity than other solvents with MIC ranged from 25 mg/ml to 50 mg/ml and MBC ranged from 50 mg/ml to 100 mg/ml. These results suggest that paw paw leaf extract is recommended as a diarrhoea disease remedy.*

Keywords— *Antimicrobial activity, Carica papaya, diarrhoea.*

I. INTRODUCTION

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 (1). 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 (2). 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. 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 (3).

Carica papaya belongs to the family Caricaceae. It has the following common names; pawpaw tree, papaya, papayer, tinti, pepol, chich put, fan kua, wan shou kuo, kavunagaci, kepaya etc. The parts that are usually used include the leaves, fruit, seed, latex, and root. The plant is described as a fast growing, erect, usually unbranched tree or shrub, 7-8 m tall with copious latex, trunk of about 20 cm in diameter. The plant is also described in a documented property forms and it act as analgesic, amebicide, antibacterial, cardiogenic, cholagogue, digestive, emenagogue, febrifuge, hypotensive, laxative, pectoral, stomachic and vermifuge. It is distributed throughout Asia, Nigeria etc (4). *Carica papaya* contains many biochemically active compounds. Two important compounds are chymopapain and papain, which are supposed to aid in digestion. Papain is used in the treatment of arthritis. The leaves of *Carica papaya* is used as soap substitute which are supposed to remove stains. The papain, the proteolytic enzyme has a wealth of industrial uses. It has milk-clotting (rennet) and protein digesting properties. Active over a wide pH range, papain is used in medicine, combating dyspepsia and other digestive disorders. In liquid preparations, it has been used for reducing enlarged tonsils. Nearly 80% of American beer is treated with papain, which digests the precipitable protein fragmented and then the beer remains clear on cooling. Papain is also used for degumming natural silk. But most of the papain imported in the U.S is used for meat-tenderizers and chewing gums.

Also used to extract the oil from tuna liver cosmetically, it is used in some dentifrices, shampoos and face-lifting preparations. Use to clean silks and wools before dyeing and to remove hair from hides during tanning (5). It is also used in the manufacture of rubber from latex (6). Recently, FDA has cleared chymopapain for intradiscal injection in patients with documented herniated lumbar inter-vertebral discs whose signs and symptoms have not responded to conservative therapy over an adequate period of time.

Papaya is a polygamous species and it is difficult to identify a plant whether it is male, female or hermaphrodite. *Carica papaya* is a lozenge tropical fruit, often seen in orange – red, yellow – green and yellow – orange hues, with a rich orange pulp. Papaya (*Carica papaya* Linn) is commonly known for its food and nutritional values throughout the world. The medicinal properties of papaya fruit and other parts of the plant are also well known in traditional system of medicine. Each part of papaya tree possess economic value when it is grown on a commercial scale (7). The leaves of the papaya plants contain chemical compounds of karpain, substance which kills microorganisms that often interfere with the digestive function (8). Papaya leaf-extracts have phenolic compounds, such as protocatechuic acid, p-coumaric acid, 5, 7-dimethoxycoumarin, caffeic acid, kaempferol, quercetin, and chlorogenic acid (9 and 10). During the last few decades, considerable progress has been achieved regarding the therapeutic properties of papaya. Recently, (11) had screened 13 Brazilian medicinal plants for antimicrobial activity against bacteria and yeasts. Antimicrobial is a substance that acts to inhibit the growth of harmful microorganisms or acts to destroy them, such as bacteria, virus, fungi, and protozoa. The discovery and development of antibiotics are among the most influential and successful achievements of modern science and technology for the control of infectious diseases. However, the rate of resistance of pathogenic microorganisms to conventionally used anti-microbial agents is increasing with an alarming frequency [12,13 and 14]. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy while there are some advantages of using medicinal plants, such as often fewer side effects, better patient tolerance, relatively affordable treatment, profound therapeutic benefit, acceptance due to long history of use and being renewable in nature. For these reasons, researchers are increasingly turning their concentration to herbal products, looking for new leads to develop better drugs against multiple drug resistant microbial strains. Herbal medicine is still the stronghold of about 75-80% of

the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents [15].

(16) observed that the inhibitory action of the plant extracts could be attributed to the presence of the phytochemical constituents in the plant extracts such as alkaloid, flavonoid and saponin.

The objective of the study was to determine the antimicrobial activity, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of this plant extract on the tested organisms causing diarrhoea.

II. MATERIALS AND METHODS

2.1 Plant collection:

Carica papaya leaf was collected from Lilo town in Ihiala L.G.A of Anambra State, Nigeria. The plant was identified and authenticated in the Department of botany, Nnamdi Azikiwe University, Awka Nigeria where the sample was deposited. The leaf spread out and dried on a clean surface under a shade at room temperature to exclude direct Sunlight in order to prevent the active constituents of the leaf from being degraded due to photochemical reactions. It was air dried for about eight days after which, it was observed to be dried. The dried leaves were gathered, and crushed with grinder. The powder was weighed using an electric weighing balance by Kern ALS 220 – 4. The powder was then stored in an air tight bag at room temperature and used for further extraction.

2.2 Preparation of plant extract

The ground leaf was prepared in three ways to get the extracts.

2.2.1 Aqueous extract (Maceration Method)

Maceration method was used for aqueous extraction and powdered leaf of *Carica papaya* was used. 150 g of the plant was weighed and put in 375 ml of distilled water and allowed to stand for 48 hrs, agitate or shake for 45 mins. The extract was filtered using British standard mesh filter and first muslin cloth and concentrated by using air drying under constant air current and water bath at 50 °C. The extract was then transferred into a clean container and stored in the refrigerator until required for use.

2.2.2 Organic solvent extraction by maceration

This was carried out at Pharmacognosis Department, Faculty of Pharmaceutical Sciences, Agulu. 150 g of the plant sample was transferred into 1000 ml volumetric flask, then 375 mls of solvent (methanol and n-hexane) were added. This was covered and allowed for 48 hrs with continuous shaking, filtered and transferred to

rotary evaporator for concentration. The extract was then transferred into a clean container and stored in the refrigerator until required for use.

2.2.3 Extraction by Soxhlet method

This method was carried out by continuously extracting a sample with a non polar organic solvent for about 4-6 hrs.

III. ANTIMICROBIAL SCREENING OF PLANT EXTRACTS.

From the stored extracts in the refrigerator, the concentrated aqueous extracts of different plants were weighed 1200 mg of extract (1.2 g) as the stock. The extract was dissolved in 3 mls of distilled water to obtain 400mg/ml as our interest. This was done for aqueous extracts of the various plants.

1200 mg (1.2 g) of methanol and n-hexane extracts of the plants were weighed and dissolved in 3 mls of DMSO to make a concentration of 400 mg /ml.

3.1 Control Organisms used for Antimicrobial screening of Plants.

Standard organisms were used for the antimicrobial / antifungal sensitivity testing.

Four of these organisms were typed organisms and were collected from Department of Pharmaceutical Microbiology, Faculty of Pharmaceutical science, Agulu, Nnamdi Azikiwe University. The organisms were subcultured in different selective media for colony morphology confirmation of the typed organisms. All the organisms were re-confirmed through biochemical tests: catalase, coagulase, motility, indole, urease and Triple sugar iron agar (TSI).

<i>Salmonella typhi</i>	NCTC 10950
<i>E.coli</i>	NCTC 10418
<i>Staphylococcus aureus</i>	NCTC 6571
<i>Shigella dysenteriae</i>	ATCC 14420
<i>Candida albican</i>	

These organisms were control organisms and were stored in agar slants in a refrigerator at 2-4 °C until used.

Prior to use, these organisms were sub-cultured on Nutrient agar plates, or Sabouraud dextrose agar plates at 37 °C for 24 h.

3.2 Determination of Susceptibilities of Organisms to Crude Extracts

Prior to testing, each organism was sub cultured from the nutrient agar slope (storage system) into a nutrient agar plate. This was incubated at 37°C for 24 hrs. After 24 hrs incubation, a colony of each tested organism was inoculated into 5 mls of sterile Nutrient broth and incubated at 37°C for another 24 hrs. Thereafter, turbidity was checked.

The turbidity was adjusted to 0.5 Macfarland standard (see appendix 2 for preparation) and diluted to obtain a final turbidity in approximately 1×10^8 cfu / ml.

Muller Hinton agar was used for bacteria while Sabouraud dextrose agar was used for fungal cultivation. These media were sterilized in an autoclave at 121°C (15 lbs pressure) for 15 min before use. Petri dishes were sterilized in a hot air oven at 175°C for 1hr and was labelled appropriately.

3.3 Agar Diffusion Method :

From the first concentration (400 mg /ml) that was gotten from the stock i.e 1200 mg extract dissolved in 3 mls, further doubling dilution was prepared to give 1:200, 1:100, 1:50, 1:25, 1:12.5, 1:6.25, 1:3.125. Then, 0.1 ml of broth culture of each tested organism or fungi was placed at the centre of a sterilized petri dish and 20 ml of prepared Muller Hinton Agar or Sabourand's dextrose agar poured into it. The dish was swirled gently to ensure even distribution of the bacteria or fungi and the mixture was then allowed to gel. When gelled, six wells of 7 mm in diameter were bored in each petri dish using a sterile cork borer and each well was labeled appropriately for each crude extract or dilution of crude extract, the wells were carefully filled with 2 drops of a 2 ml pipette of both stock solutions(crude extracts) and different dilutions of the extracts, which is equivalent to 0.04 mls starting with the highest dilutions, the control drugs were added. DMSO, Methanol and conventional antibiotic (ciprotab) were used as controls. Ciprotab was used at a concentration of 200 mg/ml. This was achieved by dissolving 500 mg of the tablet in 2.5 ml of sterile water. The plates were kept for 30 mins on the bench for diffusion of the extract to take place before incubation. The dishes were incubated at 37°C for 24 hrs and observed for inhibition. The fungi were inoculated in Sabourand dextrose agar and incubated at room temperature (25°C) for 24 - 48 hrs. The zones of inhibition were measured and the results noted. This was done for aqueous, n-hexane and methanol extracts of all the plants in the tested organisms. The whole process was repeated in triplicate.

3.4 Agar Dilution Method:

1200 mg /ml(1.2 g) of the extracts each was weighed as stock solution, Muller Hinton agar and Sabourand dextrose agar was prepared. Then, using formular:

$$C_1 V_1 = C_2 V_2$$

Because we want to get 400 mg /ml as first dilution.

Where ,

C_1 = Concentration of stock (1200), V_1 = Unknown, C_2 = 400 mg / ml (our interest), V_2 = Final volume of agar to prepare (5 mls).

It was allowed to gelled, the petri dish was divided, then from the adjusted 0.5 Macfarland broth culture of tested organism, with a loopful of diluted tested organism was streak with wire loop on top of the gelled mixture of extract and agar. Incubated at 37°C for 24 h for bacteria and at room temp for fungi. Observed for growth or absence of growth. Presence of growth was indicated using positive (+) sign or negative (-) sign. From here, the tentative minimum inhibitory concentration (MIC's) was obtained, that was the last or minimum dilution of the extracts which inhibits the visible growth of organisms. Also the tentative minimum bactericidal concentration (MBC) was obtained, that was the last or minimum dilution of the extracts in which there is no growth after subculture onto fresh media. These were indicated using (-) sign.

3.5 Minimum Bactericidal Concentration (MBC)

From the tubes showing no visible sign of growth in MIC determination, test microorganisms were inoculated onto sterile nutrient agar plates by streak plate method. The plates were then incubated at 37°C for 24 hrs. The least concentration that did not show growth of test organisms after subculture was considered as the MBC.

IV. RESULTS

The antimicrobial activities of the crude n-hexane, methanol and aqueous extracts of *Carica papaya* against test organisms were shown in Figures 1 to 3 using agar well diffusion method by measuring the diameters of growth inhibition zones in triplicate. From the results crude methanol and aqueous extracts of *Carica papaya* had no inhibitory effect on *Candida albicans* (0±0) but n-hexane extracts of *Carica papaya* had antifungal activity with mean ±SD zone diameter of (5±1). In Figure 1, n-hexane extract of *Carica papaya* had no inhibitory effect on *E.coli*(0±0) but Methanol and Aqueous extract of *Carica papaya* had activities on *E.coli* (5.33±0.57) at 400mg/ml. Also the Methanol crude extract of *Carica papaya* had no activity in all the organisms tested except in *Candida albicans*.

Table 1 shows the minimum inhibitory concentrations (MBCs / MFCs) of different extracts of *Carica papaya* on test organisms. The Aqueous extract show that the MIC_s of *E.coli* and *Shigella dysenteriae* were 100mg/ml with MBC_s of 200 mg/ml and Methanol extract show that MIC of *Shigella dysenteriae* was 25 mg/ml with MBC of 50 mg/ml, MIC of *S.aureus* was 50 mg/ml with MBC of 100 mg/ml. N-hexane extract of *Carica papaya* also show that the MIC of *S.aureus*, *E. coli* and *Shigella dysenteriae* were 25 mg/ml with MBC_s of 50 mg/ml. The MIC_s of *Candida albicans* was 50 mg/ml with MFC of 100 mg/ml.

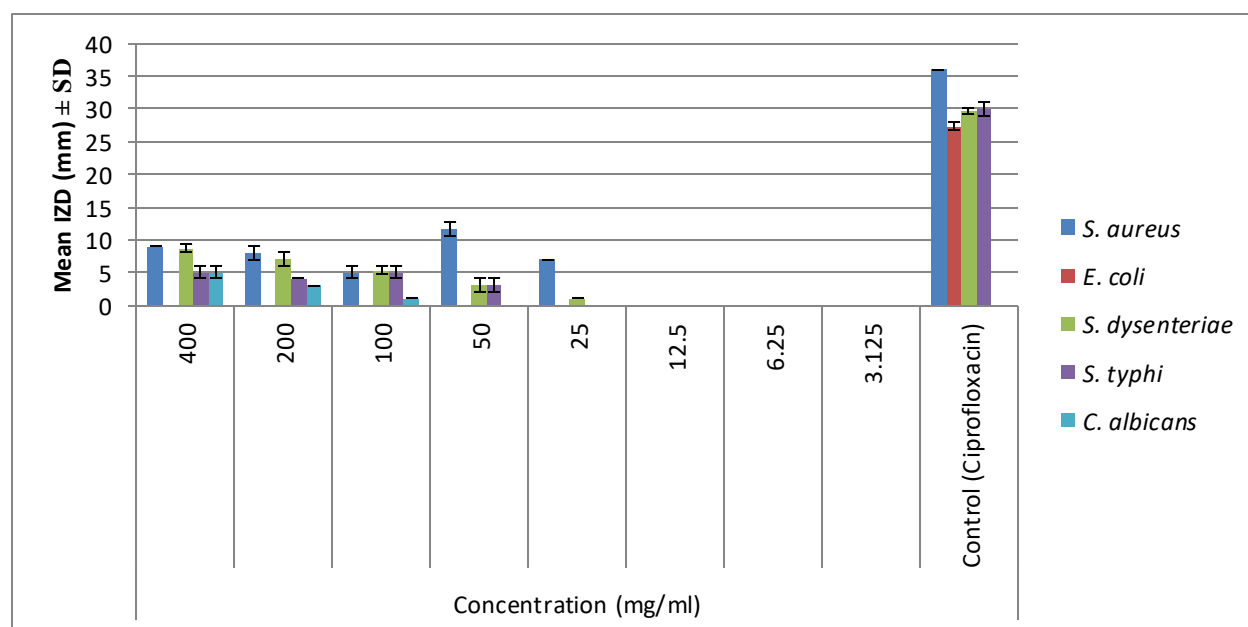


Fig.1: Antimicrobial activity of the crude n-hexane extract of *Carica papaya* leaves showing the mean inhibition zone diameters and standard deviation produced against test organisms.

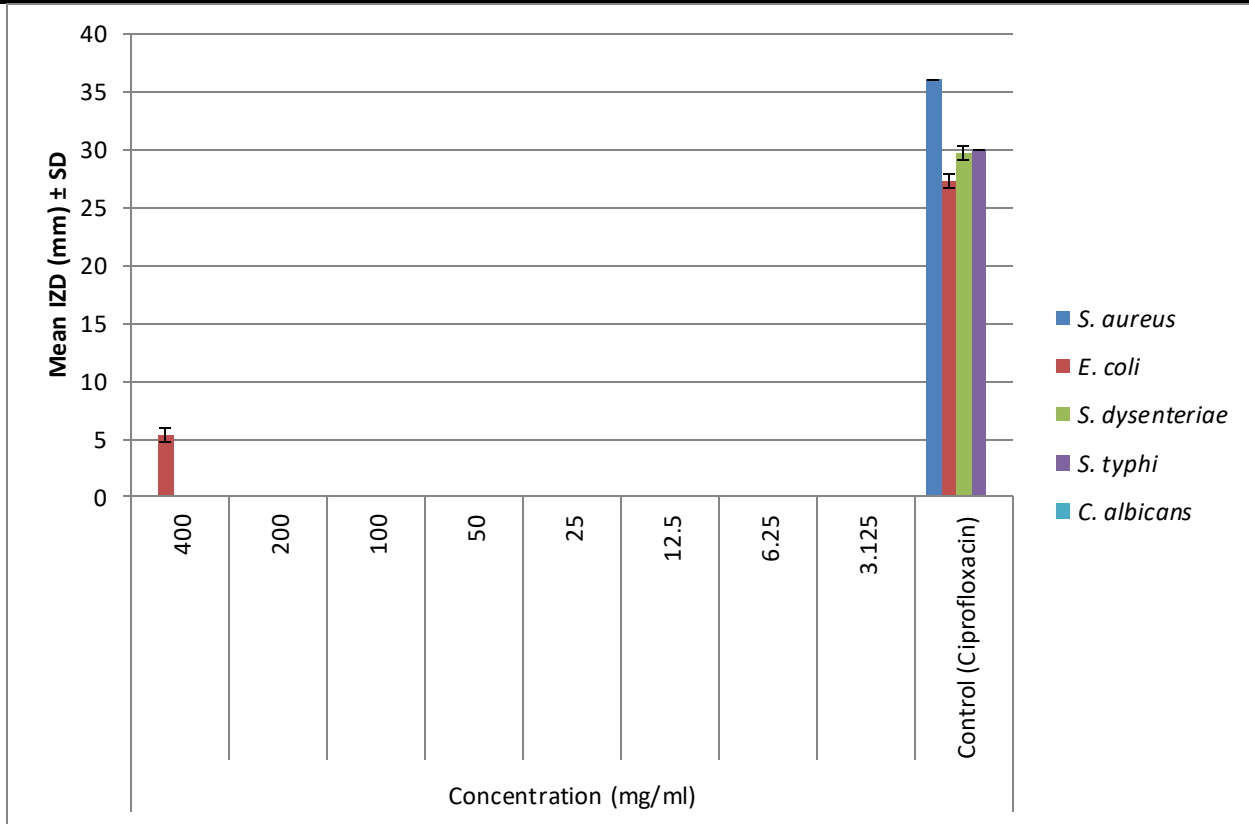


Fig. 2: Antimicrobial activity of the crude methanol extract of *Carica papaya* leaves showing the mean inhibition zone diameters and standard deviation produced against test organisms.

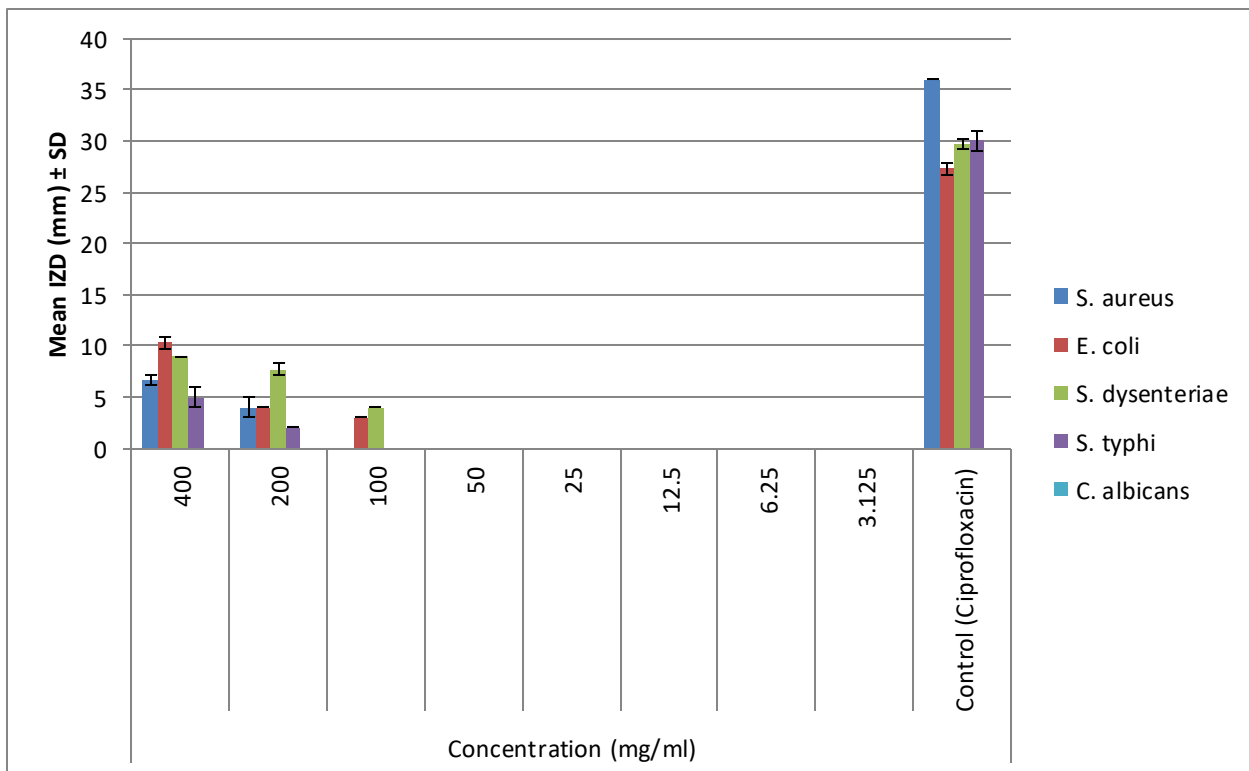


Fig. 1: Antimicrobial activity of the crude aqueous extract of *Carica papaya* leaves showing the mean inhibition zone diameters and standard deviation produced against test organisms.

Table 1: Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal/Fungicidal Concentrations (MBCs/MFCs) of extracts of *C. papaya* on test organisms.

Test Organisms	Aqueous		Methanol		N-Hexane	
	MICs	MBCs/MFCs	MICs	MBCs/MFCs	MICs	MBCs/MFCs
<i>S. aureus</i>	200	400	50	100	25	50
<i>E. coli</i>	100	200	100	200	25	50
<i>S.dysenteriae</i>	100	200	25	50	25	50
<i>S. typhi</i>	200	400	100	200	50	100
<i>C. albicans</i>	-	-	-	-	50	100

V. DISCUSSIONS

This study evaluated the antimicrobial effect of n-hexane, methanol and aqueous extracts of *Carica papaya* (paw paw) leaf against bacterial and fungal agents that causes diarrhoea. The results of the present study showed that the n-hexane extract of *Carica papaya* had significant antimicrobial effects.

The antimicrobial effect observed against the test organisms may also be as a result of these bioactive components present in the crude extract as reported by (17).

The results of the antimicrobial screening tests revealed that the crude methanol extract of *Carica papaya* were devoid of antifungal activities in- vitro as no zone of inhibition was observed on the culture plates.

The crude aqueous extract of *Carica papaya* root produced antibacterial effects but had no antifungal effect on *Candida albicans*.

The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the plant on the test organisms varied, showing that the effect of the plant extracts differed from one organism to the other.

The n-hexane extract of *Carica papaya* had more activity than other solvents with MIC ranged from 25 mg/ml to 50 mg/ml and with MBC ranged from 50 mg/ml to 100 mg/ml. However, the present study revealed that n-hexane was the best extracting solvent for *Carica papaya*. (18) had earlier reported that the percentage recovery from plants were dependent on the type of solvent used. The n-hexane extract of *Carica papaya* had more activity than other solvents with MIC of 25 mg/ml to 50 mg/ml and with MBC of 50 mg/ml to 100 mg/ml. These results clearly confirm that *Carica papaya* leaf is effective alternative therapy against microbial agents that cause diarrhoea disease.

VI. CONCLUSION

We conclude that the *Carica papaya* leaf extracts have a significant antimicrobial activity against diarrhoea causing agents. The demonstration of antimicrobial activity of

Carica papaya may help to discover new chemical classes of antibiotic substances that could serve as selective agents for diarrhoea disease control.

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