Asian Journal of Fisheries and Aquatic Research

Volume 26, Issue 8, Page 83-94, 2024; Article no.AJFAR.119884
ISSN: 2582-3760

Comparative Studies on Antibacterial Potentials of *Cassia fistula* and *Carica papaya* Leaf Extract against Selected Fish Pathogens

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Author's contribution

Author OJA conceptualized the study, did data curation, formal analysis, investigation, performed methodology and wrote the draft of the manuscript.

Article Information

DOI: https://doi.org/10.9734/ajfar/2024/v26i8798

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/119884

Original Research Article

Received: 05/05/2024
Accepted: 09/07/2024
Published: 12/08/2024

ABSTRACT

Aim: To investigate the antibacterial efficacy of *Cassia fistula* and *Carica papaya* leaf extracts against selected fish pathogens such as *Aeromonas hydrophila, Vibrio parahaemolyticus, Staphylococcus aureus, Proteus mirabilis* and *Micrococcus luteus,*
Study Design: To carry out the antibacterial sensitivity studies of *C. fistula* and *C. papaya* leaf extracts using one way analysis of variance.
Place and Duration of Study: Department of Biological Sciences, Bells University of Technology, Ota, Ogun State, between November 2022-April, 2023
Methodology: Leaves were subjected to aqueous extraction, and concentrated at 50^0C in hot air oven. The extracts were screen for biomolecules responsible for antibacterial activities. Antibacterial sensitivity test was carried out on bacteria pathogens using agar well diffusion technique.
Results: Results showed that *C. fistula* leaf extract was more effective against *A. hydrophila* (20.00^b ± 2.31 mm) *and P. mirabilis* (17.00^b ± 1.15 mm) than *C. papaya* leaf extract. (14.00^c ± 2.31 mm and

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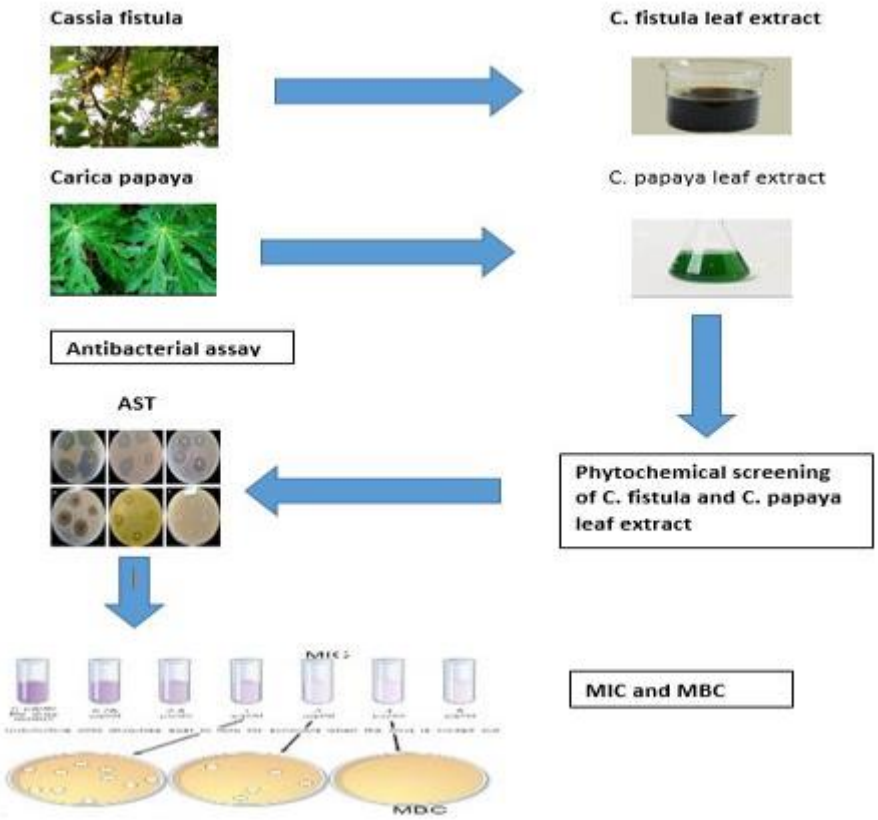
Cite as: *Abiodun, Olugbojo Joseph. 2024. "Comparative Studies on Antibacterial Potentials of Cassia Fistula and Carica Papaya Leaf Extract Against Selected Fish Pathogens". Asian Journal of Fisheries and Aquatic Research 26 (8):83-94. https://doi.org/10.9734/ajfar/2024/v26i8798.*

15.00^{c} ± 1.15 mm), while *C. papaya* was only effective against *V. parahaemolyticus* (17.50^{b} ±1.73 mm) than *C. fistula* (15.50^{c} ± 0.58 mm). The results depicted significant difference (P<0.05) in the inhibition zone among *A. hydrophila*, *V. parahaemolyticus* and *P. mirabilis*, except *S. aureus* (12.50^{b} ± 1.73 mm) and *M. luteus* (11.50^{b} ± 0.58 mm) (P>0.05). Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations confirmed that *C. fistula* was more effective on *A. hydrophila* than *C. papaya* at a lesser dose with a marked difference (P>0.05).

Conclusion: *C. fistula* leaf extract exhibited higher antibacterial efficacy than *C. papaya,* showing that it is more effective against all tested bacteria except *V. parahaemolyticus.* This implies that *C. fistula* is an excellent antibacterial agent with capacity to destroy a wide range of bacteria than *C. papaya*. Therefore, it can be used to treat fish diseases and pond water against bacteria pathogens, including multidrug resistant bacteria.

GRAPHICAL ABSTRACT



Keywords: Antibacterial efficacy; fish pathogens; phytochemicals; multidrug resistant bacteria; plant aqueous extract.

1. INTRODUCTION

Aquaculture can be defined as the farming of aquatic organisms in various aquatic environments which include oceans, lakes, ponds, streams and rivers [1]. Although aquaculture practice has been in existence for over 4000 years, its industrial exploitation began in the mid twentieth century [2].

Over the past decades, aquaculture has contributed immensely to global food production [3]. Generally, fish production contributes to one-fifth of all animal protein in human diet [4]. Studies have also shown that fish accounted for about 17 percent of animal protein, and 7 percent of all proteins consumed by the global population [5]. In Nigeria, fish consumption accounts for over 40% of the protein sources consumed daily [6]. Beyond being an energy source, the dietary contribution of fish is significant in terms of high-quality, and ease of digestion compared to other animal protein. However, the invasion of

pathogenic bacteria on fish has limited its productivity and thus reduced the availability of fish protein to the teeming population. Gram positive and gram negative bacteria pathogens that can cause fish diseases includes: *A. hydrophila,* which causes motile Aeromonas septicemia; *V. Parahaemolyticus* which causes acute hepatopancreatic necrosis; *Yesinia rukeri* which causes enteric red-mouth diseases (Yesinosis), a chronic bacteria of intensively cultured fish; *Streprococcus iniae* and *S. agalactiae* which are responsible for Streptococcosis; *Edwasiella piscida* and E*. ictaluri*, also responsible for enteric septicemia of catfishes; *Flavobacterium Branchiophilium*, which causes bacteria gill diseases (Flavobacteriosis) and several other bacteria pathogens which are responsible for various fish diseases [7,8]. Some of these bacteria are opportunistic while others are obligatory [9].

Moreover, the use of antibiotics has not yielded much results in controlling microorganisms that cause fish diseases, on the contrary, pathogens continued to develop resistance. In addition, it should not be ignored that the intensive use of antibiotics may have dangerous consequences due to their toxic effects on fish and the aquatic environment. To combat this problem, there is a need to focus on alternative sources of antibiotics as the pathogenic microbes are also gaining resistance against standard antibiotics [10]. Bacteria have the genetic ability to transmit and acquire resistance to drugs which are supposed to serve as therapeutic agents [11], and the increase in the number of drug resistant bacteria is no longer matched by discoveries of new drugs to treat variant infections [12]. In view of these, there is a need to evaluate and exploit the medicinally valuable plants against fish pathogens. Semwal et al. [13] reported that medicinal plants are easily available, very efficient, eco-friendly and usually show instant effect against pathogens. Their abundant availability also makes this approach to be highly sustainable.

Cassia fistula belongs to the family *fabaceae*. It is usually referred to as “Golden shower”. It is native to India, amazon and Sri lanka, and spread throughout various countries such as Mexico, China, Mauritius, Africa, and West Indies. *Cassia fistula* plants are used as ornamental and shade tree around several residential areas and institutions [14]. Perhaps, part of its use in this manner is to make it easily accessible due to its medicinal purpose.

Cassia fistula exhibit medicinal properties and has been in used due to its various therapeutic potencies [15]. It is a rich source of tannins, flavonoids and glycosides which are of high medical and nutritional importance. It is also rich in carbohydrates, Linoleic, Oleic, and Stearic. Flower pollen contains phenylalanine, methionine, glutamic acid and proline. Leaf of *Cassia fistula* mainly contains Oxalic Acids, Tannins, Oxyanthraquinones, Anthraquinones Derivatives. Fruit of *Cassia fistula* contains Rhein Glycosides Fistulic Acids, Sennosides A B, Anthraquinones, and Flavanoid-3-ol-derivatives. Ceryl Alcohol, Kaempferol, Bianthraquinone Glycosides, Fistulin, Essential Oils, Volatile Components, Phytol (16.1%), 2-Hexadecanone (12%), Crystals, and 4-Hydroxy Benzoic Acids [16,17].

Carica papaya, also known as ‘Pawpaw’ (common name), belongs to the family: *Caricaseae*. It is native to Africa, Central America, South of Mexico, and India. It has gained several applications due to its medicinal properties. *C. papaya* is a perennial plant, mostly without branches; has smooth stem and long-leaf stalk. It can grow as tall as 20 m height [18]. Different parts of *C. papaya* plant have been used for several therapeutic purposes. This include: fruit, bark, roots, seeds, peel, pulp, and leaf. It is also a good source of Vitamins A, B and C. It is fairly rich in calcium and iron [19]. It contains enzyme papain, which support digestion, and can be used for the treatment of ulcers. It is a good antimicrobial agent and has been effectively used against gram-negative bacteria at higher doses [20]. Its seed extract contains benzyl iso-thiocyanate, which is both bactericidal, and fungicidal at a single effective dose of 25–30 mg [21]. *Papaya* is a good antioxidant and can be used to neutralize free radicals generation and thus prevent pathogenesis [22]. Latex is one of the most important constituents of papaya which contains papain, glycyl-endopeptidase, chymo-papain and Caricain, and their abundance depend on different parts of papaya plant [23].

Current findings shows that pawpaw leaf has several active constituents such as ascorbic acid, alpha-tocopherol, chymopapain, glucosinolates, and papain which can improve blood antioxidant properties, and has been used for the treatment of various diseases [24,25]. Several studies have been conducted on isolation and characterization of the bioactive ingredients in pawpaw leaves. Nugroho et al. [26] reported that phytochemicals

such as alkaloids, saponins, tannins, flavonoids and glycosides are present in young pawpaw leaves, which are responsible for its therapeutic properties [21].

C. fistula and *C. papaya* were chosen, because they are rich sources of important biomolecules which are absent in several other plants, and yet have not been fully explored. In addition, they are easily accessible without incurring any cost, which make it easy for fish farmer to use for treating their fish ponds, and also as inclusion in fish diets to combat various bacteria fish diseases.

The focus of this study is to evaluate and compare the antibacterial activity of *Cassia* fistula leaf extract with *Carica papaya* leaf extracts in some selected fish pathogens, and to determine their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

2. MATERIALS AND METHODS

2.1 Collection and maintenance of the organisms used

The experimental bacteria used were pathogens already isolated from *C. gariepinus*. The isolates include *A. hydrophila, V. parahaemolyticus, S. aureus, P. mirabilis* and *M. luteus*. For easy identification and prompt growth, selective media were used to obtain pure culture of the bacteria isolates. *A. hydrophila,* was cultured on Ampicillin sheep blood agar (ASBA), *V. parahaemolyticus* was cultured on Thiosulphate citrate bile salt sucrose agar (TCBS), *S. aureus* and *M. luteus* were cultured on Mannitol salt agar (MSA)*, P. mirabilis* was cultured on Salmonella Shigella agar (SSA).

A. hydrophila was observed as haemolytic bacteria lysing the red blood cell with a clear zone (Beta haemolysis), *S. aureus* appeared yellow on Mannitol salt agar while *M. luteus* appeared as small red colony, *V. parahaemolyticus* appeared green on TCBS*. P. mirabilis* was identified as an actively motile organism, colorless with black center. They were also subjected to microscopic examination and biochemical test to ascertain their identity [27,28]. They were then inoculated on Nutrient agar slants, incubated at 37°C for 24 hours and kept as stock cultures in the refrigerator at 4°C.

2.2 Collection and Identification of Plant Materials

Fresh and healthy leaves of *C. fistula* were collected from Covenant University campus while *C. papaya* leaves were collected within Bells University of Technology (Bellstech) campus, Ota. The leaves were certified by ethno-botanist in the Department of Biological Sciences, Bells University of Technology, Ota, Ogun state.

2.3 Preparation of *C. fistula* (Golden shower) and *C. papaya* Leaf Extract

The procedure for the preparation of the leaf extract was carried out separately for each of the leaf samples (*C. fistula* and *C papaya*). 10 g of each of the plant leaves was washed, and air dried at room temperature for about two weeks. The leaves were cut into small sizes, washed thrice with deionized water, and boiled with 100 ml of deionized water at 70˚C in a hot plate (Stuart- US 150) for 1hr. After boiling, the leaf extracts were filtered (using Whatman No.1 filter paper), and the aqueous filtrates were concentrated (by evaporation) at 50°C using hot-air oven. The concentrated filtrates were kept in the refrigerator at 4^0C for further use [21,29].

Aqueous extraction method was employed to ensure benign environment during extraction, and to prevent toxicity, which is the main purpose of this research. It is also cheaper and easier to adopt or recommend to fish farmers than other expensive methods. It does not necessarily requires evaporation when applying to treat fish pond or diseased fish, unlike other extraction agents (chloroform, methanol, petroleum ether, N-hexane etc.) which will require evaporation before use, and may still have traces of toxicity.

2.4 Phytochemical Screening of *C. fistula* and *C. papaya* Leaf Extract

Naturally occurring biomolecules from *C. fistula* and *C. papaya* leaf extracts were analyzed. This includes Phenols, Alkaloids, Saponins, Steroids, Flavonoids, Glycosides, Terpenoids, Proteins, and Carbohydrates**.** The following standard procedures were employed during the phytochemical screening according to Ghotekar et al. [30] and Adetunji et al. [31]. Those that were present were recorded, and those that were not present were also documented.

2.4.1 Test for saponins

Distilled water (5 mL) was added to crude extract in a test tube. The mixture was shaken vigorously for two minutes. Persistent foaming on shaking indicated the presence of saponins.

2.4.2 Test for alkaloids

Crude extract (3 mL) was mixed with 1% HCl (2 mL), and then heated for 20 minutes on water bath. The mixture was filtered after cooling. Few drops of Mayer‘s and Wagner‘s reagents were added. The presence of alkaloids was indicated by the turbidity of the resulting precipitate.

2.4.3 Test for phenols

To the mixture of ethanolic (5 mL) and aqueous extract in a test tube, 2 drops of 5% $FeCl_3$ were added. A greenish precipitate showed the presence of phenols.

2.4.4 Test for tannins

10% of freshly prepared KOH (1 mL) was added to the aqaueos extract (1 mL). The appearance of dirty white precipitate showed the presence of tannins.

2.4.5 Test for steroids

5 drops of H_2SO_4 was added to 1ml of crude extract. Red colouration showed the presence of steroids.

2.4.6 Test for flavonoids (Alkaline reagent test)

To 2 mL extract, 2 mL of 2% NAOH solution was added. Deep yellowish colouration which turned colourless when a few drops of diluted acid was added signified the presence of flavonoids.

2.4.7 Test for glycosides

Crude extract (1 mL) was mixed with chloroform (2 mL). Then, 2 mL of concentrated H_2SO_4 was carefully poured to the mixture and shaken gently. Reddish brown colouration showed the presence of glycoside

2.4.8 Test for terpenoids

Crude extract (2 mL) was dissolved in chloroform (2 mL) and then evaporated to dryness. Concentrated H_2SO_4 (2 mL) was added to the resulting solid and heated for 2 minutes. The appearance of greyish colouration indicated the presence of terpenoids.

2.4.9 Test for carbohydrate

Molisch‘s reagent was mixed with crude extract (2 mL) and shaken vigorously. Then concentrated H_2SO_4 (2 mL) was carefully added along the side of the test tube. Purple ring at the interphase of the test tube confirmed the presence of carbohydrates (Molisch‘s test).

2.4.10 Test for coumarins

Concentrated HCl and a few drop of Echrlich reagent was mixed with 2 mL crude extract. Appearance of yellow color showed the presence of coumarins

2.4.11 Test for betacyanin

To the crude extract (or filterate), a few drops of NAOH was added, the conversion of the extract to a dull yellow colour indicated the presence of betacyanin. When a few drop of concentrated HCL was added again, the colour disappeared. This confirmed the presence of betacyanin.

2.5 Antibacterial Assay

Antibacterial activities of *Cassia fistula* and *Carica papaya* leaf extracts were conducted using agar well diffusion technique [32,33], against the test isolates: *A. hydrophila, V. parahaemolyticus, S. aureus, P. mirabilis*, *and M. luteus.* Mueller Hinton agar plates were inoculated with suspensions of the test isolates from the pure cultures. Turbidity of the inoculum of the five bacterial fish pathogens were compared with 0.5 McFarlan’s standard and each of the isolates was spread all through the surface of the sterile Mueller Hinton agar plates with the aid a sterile cotton swab to ensure they were spread uniformly. With the use of a sterile 5mm cork borer, three wells were made on the inoculated agar, one for each of the plant extract while the third well was used for Ofloxacin (control). They were filled with 1ml of 200 mg/ml of each of the extracts, and the third was filled with 200 mg/ml of Ofloxacin (control). To allow even diffusion of the plant extract into the agar medium, they were kept in the refrigerator for one hour and thereafter were incubated at 37°C for 18-24 hours. After the incubation, the diameter of inhibition zone around each well was measured to the nearest millimeter. All experiments were conducted in two replicates, and the results were recorded accordingly.

2.6 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Broth dilution method was used to prepare the plant extract for the determination of the MIC and MBC as described by Wayne, and Krishna et al. [34,35]. Muller Hinton (MHB) broth was prepared according to the manufacturer's instructions. Stock solutions containing 100 mg/mL of each of the plant extracts were used for the determination of the MIC. Antibacterial potencies of each of the plant extracts against five clinically isolated Gram-positive and Gram-negative bacteria *(A. hydrophila, V. Parahaemolyticus, P. mirabilis,* and gram positive *S. aureus and M. luteus)* were determined by inoculating different concentrations of the plant extracts (prepared from the stock solution) on each of the bacterium. Bacterial suspensions of each of the experimental organism were prepared using 0.5 McFarland turbidity standard. 50µL of the prepared suspension was inoculated into each of the serially diluted tubes containing the plant extract at various concentrations. Negative and positive control tubes were also prepared to monitor and ensure that the entire procedure and condition is sterile and no environmental organism interfere with the result. The negative control test tube contained sterile broth, while the positive control test tube contained the inoculum and Muller Hinton broth (MHB) without plant extract. After incubating for 24 hours at 37°C, the visual turbidity were checked on each test tube from which the MIC values were recorded. The lowest concentrations of the plant extract which show visual inhibition on the bacteria growth were taken as the MIC [35]. This was conducted for each of the test organisms.

For the MBC, 0.05ml (50 µL) aliquots from each of the tubes that did not reveal any observable bacterial growth were inoculated and spread on Muller Hinton agar plates which does not contain antibacterial agents (plant extract). The plates were labelled with the same code on the dilution tube where each inoculum was taken and then incubated at 37 °C for 18-24 h. The agar plate was then examined after 24 hour of incubation to know if there were any bacterial growth. The petri dish which represent the lowest concentration of the plant extract which did not reveal any bacterial growth was taken as the Minimum Bactericidal Concentration (MBC). Thus, the MBC can be referred to as the lowest concentration of any antimicrobial agent or drug which can destroy 99.9% of the initial test bacterial population [35].

2.7 Statistical Analysis

Data were analyzed using one-way Analysis of Variance` (ANOVA), SPSS 18 (Statistical Package for the Social Sciences) and 10 Microsoft Excel. Duncan Multiple Range test (DMRT) was used to separate the means at $P \leq 0.05$.

3. RESULTS

3.1 Phytochemical Analysis of *C. fistula* and *C. papaya* Leaf Extract

Phytochemicals that were present during the screening carried out on *C. fistula*, and *C. papaya* leaf extract are shown in Table 1. They include Saponins, flavonoids, alkaloids, betacyanins, phenols, and coumarins. These are the active ingredient (Macromolecules) responsible for the antibacterial activity of the extracts. Result also showed that Saponins was found in *C. fistula* only while Alkaloids was also found in *C. papaya* but not in *C. fistula*. According to Table 1, other listed phytochemicals were not detected during the screening.

3.2 Antibacterial Sensitivity Test

The results of antibacterial sensitivity test provided in Table 2 depicted different degree of reaction by the test organisms (*A. hydrophila, V. parahaemolyticus, S. aureus, P. mirabilis*, *M. luteus*) on *C. fistula* and *C. papaya* leaf extract at 200 mg/ml by means of inhibition zone diameters using agar well diffusion method. *C. fistula* induced higher zone of inhibition on *A. hydrophila* ($20.00^{b} \pm 2.31$ mm) and *P. mirabilis* ($17.00^{b} \pm 1.15$ mm) than *C. papaya* ($14.00^{c} \pm 2.31$ mm, $15.00^{c} \pm 1.15$ mm) while *C. papaya* was more effective on *V. parahaemolyticus* ($17.50^{b} \pm 1.73$ mm) than *C. fistula* ($15.50^{c} \pm 0.58$ mm). The results also show that there is significant difference ($P<0.05$) along the column among *A. hydrophila*, *V. parahaemolyticus* and *P. mirabilis*, except *S. aureus* ($12.50^{b} \pm 1.73$ mm) and *M. luteus* ($11.50^{b} \pm 0.58$ mm) which revealed equal inhibition zone values on *C. fistula* and *C. papaya* with no significant difference ($P>0.05$). The inhibition zones displayed by each bacteria when tested with Ofloxacin (control) are significantly different ($P<0.05$) and higher than those displayed when tested with each of the

plant extracts along the column (Table 2). This shows that Ofloxacin (control) is more effective on the pathogens, except for its toxicity. On the two extracts, the result shows that *C. fistula* leaf extract exhibited a higher antibacterial efficacy than *C. papaya* leaf extract.

3.3 Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) recorded on *C. fistula* and *C. papaya* are shown in Table 3. The result revealed that *C. fistula has* higher potency above *C. papaya* on *A. hydrophila (*6.25^{b}± 0.00 mg/mL) and *P. mirabilis* (18.75^{a} ± 7.21 mg/mL) (Table 3) except on *V. parahaemolyticus (*18.75^{a} ± 7.21 mg/mL), while on *S. aureus* and *M. luteus*, both of the extract exhibited equal inhibitory effect (50.00^{a} ± 0.00 mg/mL). However, the MIC of *C. fistula* and *C. papaya* on most of the organism along the column revealed no significant difference (P>0.05), except on *A. hydrophila* which showed high significant difference (P>0.05) between *C. fistula* (20.00^{b} ± 2.31 mg/mL) and *C. papaya* (14.00^{c} ± 2.31 mg/mL), whereas between *C. fistula* (6.25^{b}± 0.00 mg/mL) and Ofloxacin (3.13^{b} ± 0.00 mg/mL - Control) there was no significant difference (P>0.05). MIC recorded on Ofloxacin (control) shows high significant difference, when compared with *C. fistula* and *C. papaya* on all bacteria along each column. Low MIC (3.13^{b} ± 0.00mg/mL) recorded when tested with Ofloxacin shows that a little dosage of Ofloxacin is enough to inhibit the growth of the test organisms. Low MIC depicted by *C. fistula* when compared with *C. papaya,* on *A. hydrophila*, and *P. mirabilis* showed that *C. fistula* is more potent on them than *C. papaya*, while on *V. parahaemolyticus C. papaya* was found to be more potent than *C. fistula.*

3.4 Minimum Bactericidal Concentration

Minimum bactericidal concentration of *C. fistula* and *C. papaya* are shown in Table 4. The result shows that *C. fistula* was more effective on *A. hydrophila* (9.38^{b} ± 3.60 mg/mL) and *P. mirabilis* (25.00^{a} ± 0.00 mg/mL) than *C. papaya* (50.00^{a} ± 0.00 mg/mL and 50^{a} ± 14.43 mg/mL respectively). Whereas on *V. parahaemolyticus*, *C. papaya* was more effective (18.75^{a} ± 7.2 2mg/mL) than *C. fistula* (25.00^{a} ± 0.00 mg/mL). *S. aureus* and *M. luteus* showed equal MBC in each case (Table 4), which means that both can be used to treat fish infected with *Micrococcus luteus* and *Staphylococcus aureus* with equal results. There was no significant difference in the MBC recorded on *C. fistula* and *C. papaya* when tested on *S. aureus*, *Proteus mirabilis*, *M. luteus*, and *V. parahaemolyticus*, whereas for *A. hydrophila* the difference is highly significant.

4. DISCUSSION

The continuous resistance of bacteria to antibiotics, and the production of all kinds of antibiotics coupled with their toxic effect on fish and water is a serious setback in aquaculture, and this has necessitated the need to seek for alternative antibacterial which are effective, eco-friendly, cheaper and non-toxic [13].

Table 1. Phytochemical analysis of *C. fistula* and *C. papaya* leaf extract

Serial Number	Phtochemicals	*C. fistula*	*C. papaya*
1	Saponins	+	-
2	Flavonoids	+	+
3	Alkanoids	-	+
4	Betacyanins	+	+
5	Phenols	+	+
6	Coumarins	+	+
7	Tannins	-	-
8	Steroids	-	-
9	Carbohydrates	-	-
10	Glycosides	-	-
11	Terpenoids	-	-

Table 2. Antibacterial sensitivity test (Mean ± SD in Millimeter)

Plant extract	*A. hydrophila*	*V. parahaemolyticus*	*S. aureus*	*P. mirabilis*	*M. luteus*
C. fistula	$20.00^{b} \pm 2.31$	$15.50^{c} \pm 0.58$	$12.50^{b} \pm 1.73$	$17.00^{b} \pm 1.15$	$11.50^{b} \pm 0.58$
P. papaya	$14.00^{c} \pm 2.31$	$17.50^{b} \pm 1.73$	$12.50^{b} \pm 1.73$	$15.00^{c} \pm 1.15$	$11.50^{b} \pm 0.58$
Ofloxacin (control)	$26.50^{a} \pm 0.58$	$27.50^{a} \pm 0.58$	$25.50^{a} \pm 0.58$	$26.50^{a} \pm 0.58$	$26.50^{a} \pm 0.58$

Concentration- 200 mg/ml; Control- Ofloxacin

Foot note: Mean ± SD with superscript of the same alphabet: 'a', 'b' or 'c' along the column depicts no significant difference (P>0.05)

Mean ±SD with superscript of different alphabet: 'a', 'b, or c' along the column depicts significant difference (P<0.05)

Table 3. Minimum inhibitory concentration (Mean ± SD in mg/mL)

Plant Extract	*A. hydrophila*	*V. parahaemolyticus*	*S. aureus*	*P. mirabilis*	*M.* luteus
C. fistula	$6.25^{b} \pm 0.00$	$18.75^{a} \pm 7.21$	$50.00^{a} \pm 0.00$	$18.75^{a} \pm 7.21$	$50.00^{a} \pm 0.00$
C. papaya	$37.50^{a} \pm 14.43$	$12.50^{a} \pm 0.00$	$50\ 00^{a} \pm 0.00$	$25.00^{a} \pm 0.00$	$50.00^{a} \pm 0.00$
Ofloxacin	$3.13^{b} \pm 0.00$	$3.13^{b} \pm 0.00$	$3.13^{b} \pm 0.00$	$3.13^{b} \pm 0.00$	$3.13^{b} \pm 0.00$

Concentration- 100 mg/ml; Control- Ofloxacin

Foot note: Mean ± SD with superscript of the same alphabet: 'a', 'b' or 'c' along the column depicts no significant difference (P>0.05)

Mean ±SD with superscript of different alphabet: 'a', 'b' or 'c' along the column depicts significant difference (P<0.05)

Table 4. Minimum bactericidal concentration (Means ± SD in mg/mL)

Plant Extract	*A. hydrophila*	*V. parahaemolyticus*	*S. aureus*	*P. mirabilis*	*M. luteus*
C. fistula	$9.38^{b} \pm 3.60$	$25.00^{a} \pm 0.00$	$75.00^{a} \pm 28.87$	$25.00^{a} \pm 0.00$	$75.00^{a} \pm 28.87$
C. papaya	$50.00^{a} \pm 0.00$	$18.75^{a} \pm 7.22$	$75.00^{a} \pm 28.89$	$37.50^{a} \pm 14.43$	$75.00^{a} \pm 28.87$
Ofloxacin	$6.25^{b} \pm 0.00$	$4.69^{b} \pm 1.80$	$4.69^{b} \pm 1.80$	$4.69\ ^{b} \pm 1.80$	$4.69^{b} \pm 1.80$

Concentration- 100 mg/ml; Control- Ofloxacin

Foot note: Mean ± SD with superscript of the same alphabet: 'a', 'b' 'c' along the column depicts no significant difference (P>0.05)

Mean ±SD with superscript of different alphabet: 'a', 'b', or 'c' along the column depicts significant difference (P<0.05)

The result of qualitative phytochemical screening showed that biomolecules such as flavonoids, betacyanins, phenols, and coumarins were present in both plant extracts while saponins was only found in *C. fistula*, and Alkanoid in *C. papaya*. This result is in agreement with the literature according to Ajiboye and Olawoyin [36], which revealed that some biomolecules may not be easily detected with aqueous extraction method but their presence could only be detected or inferred when tested on pathogens. Omidiwura [37] also explained that the effectiveness of every plant extract is a function of bioactive compounds present in them, some of which will require the use of stronger extraction agent to detect them. These compounds are known to be biologically active and therefore, aid the antibacterial activities of the plant extract [38].

Antibacterial activity of *C. fistula* and *C. papaya* leaf extract revealed that both exhibited varied degrees of antibacterial activities. However, *C. fistula* leaf extract showed higher antibacterial potential than *C. papaya*. This could be as a result of the constituent bioactive compound present in *C. fistula* which could be higher in quantity and quality than *C. papaya*; an indication that some plants have greater ability to inhibit bacterial growth than others [36].

However, the zone of inhibition as measure against *C. fistula* was low when compared with standard drug (Ofloxacin). The highest activity was recorded with Ofloxacin in both extract, this is because it is a standard antibiotic and it is in a pure state.

Minimum inhibitory concentration showed that *C. fistula* exhibited higher inhibitory ability on *A. hydrophila* and *P. mirabilis* than *C. papaya*, except on *V. parahaemolyticus where C. papaya* was found to exhibit higher potency. When examined on *S. aureus* and *M. luteus*, they both exhibited equal inhibitory ability. Likewise, minimum bactericidal concentrations show that both extracts have equal antibacterial potential on *S. aureus* and *M. luteus.* On *A. hydrophila,* and *P. mirabilis*, *C. fistula* still retain higher MBC, while on *V. parahaemolyticus, C*. *papaya* was found to exhibit higher potency than *C. fistula*, although the difference is not significant. The insignificant difference in the MIC and MBC of Ofloxacin (control) and *C. fistula* when examined on *A. hydrophila* shows that *C. fistula* has a more promising potentials to serve as antibacterial agent especially against *A. hydrophila,* than *C. papaya* which is significantly lower. This shows that, *C. fistula* and can be effectively used as a replacement for antibiotics than *C. papaya*. This result aligns with the previous literature according to indhumathy et al. [39] and Pawar et al. [40] who asserted that, apart from the common biomolecules present in both *C. fistula* and C. *papaya*, *C. fistula* is a very rich source of anthraquinones, terpenoids, reducing sugar and steroids than *C. papaya.* The higher potency of *C. fistula* than *C. papaya* was also supported by Sign et al., [21]. *C. fistula* was known to contain more biomolecules per gram, in terms of quantity and quality than *C. papaya*. In addition to the constituent biomolecules earlier mention on *C. fistula*, several literatures also proved that *C. fistula* leaf contain oxalic acids, oxy-anthraquinones and their derivatives [15] which could be responsible for its excellent antibacterial efficacy.

5. CONCLUSION

The present study confirmed that at equal concentration, *C. fistula* leaf extract was more potent than *C. papaya* leaf extract on *A. hydrophila,* and *P. mirabilis* while on *S. aureus and M. luteus* they exhibited equal efficacy. This could be due to the fact that*, C. fistula* contains certain organic derivatives in addition to the detected biomolecules which perhaps due to aqueous extraction method used, were not detected during phytochemical screening, but reflected during sensitivity test. Nevertheless, *C. papaya* was also seen to be more effective on *V. parahaemolyticus.*

In general, this finding justifies the traditional uses of plant parts for therapeutic and prophylactic purpose on fish against pathogens especially the selected and tested organisms. It also proved that plants are potential sources for production of novel drugs for the treatment of fish diseases and can also be used to treat pond water before stocking.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

HIGHLIGHTS

(1) Fish accounted for about 17% of the total animal protein, and 7% of all proteins

consumed by the global population; and in Nigeria it accounts for over 40% of the daily protein consumption.

(2) The presence of bacteria pathogens in fish and its environment is a major draw-back in aquaculture, with significant limitation in fish availability, both in quantity and quality.

(3) Antibiotics toxicity, and the emergence of multidrug resistant bacteria presupposes the need for a more effective, non-toxic and eco-friendly alternative.

(4) Plant extracts are found to be excellent and broad spectrum antibacterial agents, best alternative to toxic antibiotic drugs, especially against multidrug resistant bacteria. They are easily available, cost effective and highly sustainable.

(5) *C. fistula* leaf extract was found to be more effective against *A. hydrophila* and *P. mirabilis*, than *C. papaya* leaf extract, while *C. papaya* was only more effective on *V. paraheamolyticus*.

(6) *Generally, C. fistula* leaf extract exhibited higher antibacterial efficacy on tested bacteria than *C. papaya leaf extract*, and therefore recommended for the treatment of fish bacteria diseases and parasites, including multidrug resistant bacteria.

ACKNOWLEDGEMENT

The author sincerely appreciate the Department of Biological Sciences, Bells University of Technology, Ota for making the laboratory and other facility available for this research, and Dr. Oluwaseun Ejilude of Tuberculosis Reference Laboratory, University College Hospital, Ibadan for his support during the sensitivity studies.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/119884