Comparative studies on antibacterial potentials of *Cassia fistula* and *Carica papaya* leaf extract against selected fish pathogens

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

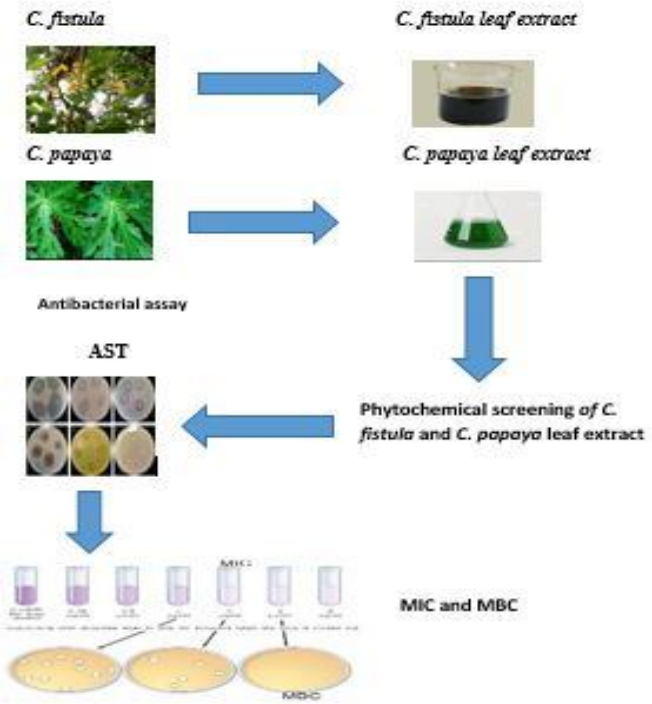
Methodology: Leaves were subjected to aqueous extraction, and concentrated at 50^0C in hot air oven. The extracts were screen for biomolecules responsible for antibacterial activities. Antibacterial sensitivity test was carried out on bacteria pathogens using agar well diffusion technique.

Result: Results showed that *C. fistula* leaf extract was more effective against *A.hydrophila*($20.00^b \pm 2.31$ mm) *and P. mirabilis* ($17.00^b \pm 1.15$ mm) than *C. papaya* leaf extract. ($14.00^c \pm 2.31$ mm and $15.00^c \pm 1.15$ mm),while *C. papaya* was only effective against*V. parahaemolyticus* ($17.50^b \pm 1.73$ mm) than*C. fistula* ($15.50^c \pm 0.58$ mm). The results depictedsignificant difference ($P<0.05$) in the inhibition zone 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) (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 alltested bacteria except*V. parahaemolyticus.* This implies that *C. fistula* is an excellentantibacterial 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.

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 shownthat 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;*Streprococcusiniae* and *S. agalactiae* which are responsible forStreptococcosis; *Edwasiellapiscida* and E. *ictaluri*, also responsible forenteric 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 availabilityalso makesthis 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 medicaland 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 effectivelyused 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 findingsshows 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 inpawpawleaves. Nugroho *et al* [26] reported that phytochemicalssuch 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).

MATERIALS AND METHODS

Collection and maintenance of organisms used

The experimental bacteriaused 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 pureculture of the bacteria

isolates.*A. hydrophila,* was cultured on Ampicillin sheep blood agar (ASBA), *V. parahaemolyticus* was culturedon Thiosulphate citrate bile salt sucrose agar (TCBS), *S. aureus* and *M. luteus*were culturedonMannitol salt agar (MSA), *P. mirabilis* was culturedon 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 motileorganism, colorless with blackcenter. 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.

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.

Preparation of*C. fistula*(Goldenshower) 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*). 10gofeach of the plant leaveswas washed,and airdriedat room temperature foraboutwoweeks. The leaves werecut into small sizes, washed thrice with deionized water, andboiledwith100mlofdeionizedwaterat70˚C for 1hr.After boiling, the leafextracts were filtered(usingWhatmanNo.1filter paper), and the aqueous filtrates wereconcentrated (by evaporation) at 50°C using hot-air oven. Theconcentrated filtrates werekept 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 farmersthan 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.

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**.** Thefollowing standard procedures were employed during the phytochemical screeningaccording 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.

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.

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.

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.

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.

Test for Steroids

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

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.

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

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.

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).

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

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.

Antibacterial assay

Antibacterial activities of *Cassia fistula* and *Carica papaya* leaf extracts were conducted using agar well diffusion technique [32, 33], againstthe 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/mlofeach 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,andthe resultswere recorded accordingly.

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 100mg/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-positiveand 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 thebacteria 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].

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 $P \leq 0.05$.

RESULTS

Phytochemical analysisof*C. fistula*and *C. papaya*leafextract

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

Table 1 Phytochemical analysisof*C. fistula*and *C. papaya*leafextract

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	-	-

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} ± 2.31 mm) and *P. mirabilis* (17.00^{b} ± 1.15 mm) than *C. papaya* (14.00^{c} ± 2.31 mm, 15.00^{c} ± 1.15 mm) while *C. papaya* was more effective on *V. parahaemolyticus* (17.50^{b} ±1.73 mm) than *C. fistula* (15.50^{c} ± 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} ± 1.73 mm) and *M. luteus* (11.50^{b} ± 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 testedwitheach 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.

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

Footnote:

Mean± SDwithsuperscriptofthesamealphabet: 'a', 'b' or 'c' along the columndepictsno significantdifference (P>0.05)

Mean±SD withsuperscriptofdifferentalphabet:'a','b, or c' along the column depictssignificantdifference(P<0.05)

MinimumInhibitory 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 (*$6.25^{b}\pm$ 0.00 mg/mL) and *P. mirabilis* ($18.75^{a} \pm 7.21$ mg/mL) (Table 3) except on *V. parahaemolyticus (*$18.75^{a} \pm 7.21$ 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.*

Table 3: **MinimumInhibitory Concentration (Mean±SD in mg/mL)**

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

Concentaration- 100 mg/ml
Control- Ofloxacin

Footnote:

Mean± SDwithsuperscriptofthesamealphabet: ‘a’,‘b’ or ‘c’ along the columndepictsno

significantdifference (P>0.05)

Mean±SD withsuperscriptofdifferentalphabet:'a','b'or'c' along the column depicts significantdifference(P<0.05)

Minimumbactericidal 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} ± 0.00mg/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 *Staphylococcus aureus, Proteus mirabilis, Micrococcus luteus*, and *V. parahaemolyticus*, whereas for *A. hydrophila* the difference is highly significant.

Table 4 **Minimumbactericidal concentration(Mean±SD in mg/mL)**

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

Concentaration- 100 mg/ml

Control- Ofloxacin

Footnote:

Mean± SDwithsuperscriptofthesamealphabet: ‘a’,‘b’ ‘c’ along the columndepictsno significantdifference (P>0.05)

Mean±SD withsuperscriptofdifferentalphabet: ‘a’,‘b’,or ‘c’along the column depictssignificantdifference(P<0.05)

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]

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. hydrophyla,* 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. hydrophyla,* 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.

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.

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.

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.

REFERENCES

[1] FAO.State of World Fisheries and Aquaculture (Spanish). Food and Agriculture Org., S. l., Rome.2016

[2] Dias JD, Simões NR, Bonecker CC.Net cages in fish farming: a scientometric analysis. *Acta LimnologicaBrasiliensia.*2012; 24: 12– 17.

[3] FAO. The state of World fisheries and Aquaculture. Rome. 2014; Pp. 223. www.fao.org/3/a-i3720e.pdf

[4] Olaniyi, WA, Omitogun, OG.Induction of Tripoloidy and Erythrocyte cell size analysis of Tripoloid African Catfish, *Clarias gariepinus* (Buchell 1822). *Animal Research International.*2014; 11 (3). 2079-2086

[5] Thompson, B and Amoroso L. Improving diets and nutrition: Food-based approach, FAO. 2015.

[6] Worldfish Centre; 2015. www.worldfishcentre/nigeria.org.

[7] Damba EP,Bichi AH, Ishaku S, AhmadMK, Buba U,Bingari MS et al Occurrence of pathogenic bacteria associated with *Clarias gariepinus*in selected Fish farms of kumbotso LocalGovernement Area of Kanostate, Nigeria. *Bayero Journal of Pure and Applied Sciences*2014; 7(2): 145 – 149. http://dx.doi.org/10.4314/bajopas.v7i2.25

[8] Hardi EH, Rudy AN, Gina S, Ria S, MaulinaA, Mira M. Identification of potentially pathogenic bacteria from tilapia (*Oreochromis niloticus)* and channel catfish (*Clarias batrachus*) culture in Samarinda, East Kalimantan, Indonesia. *Biodiveritas.* 2018; *19(2):480-488.* DOI: 10.13057/biodiv/d190215. ISSN: 1412-033X.

[9] Sarma P and Kardong D. Enzyme-producing gut bacteria of fish and its effect on Fish Health: A Review. Uttar Pradesh Journal of Zoology. DOI:10.56557/upjoz/2022/v43i153117. 2022
.

[10] Tarun A, Yash Prashar YR. A review on medicinal properties of *Carica papaya* Linn.Asian Pacific Journal of Tropical Disease. doi: 10.1016/S2222-1808(14)60617-4. 2015; 5(1): 1-6.

[11]Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, and Bezirtzoglou E. Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives.
Microorganisms. 2021; 9(10): 2041.doi: 10.3390/microorganisms9102041

[12] Linz, M.S, Mattappallil, A, Finkel D, and Parker D. Clinical Impact of *Staphylococcus aureus* Skin and Soft Tissue Infections. *Antibiotics* (Basel). 2023; 12(3): 557. doi: 10.3390/antibiotics12030557

[13]Semwal A, Kumar A, and Kumar N. A review of pathogenic *Aeromonas hydrophila* and their mitigation through medicinal herb in Aquaculture. *Heliyon*. 2023; 9 (3). E14088. Doi.
10.1016/j.heliyon.2023.e14088.

[14] Mwangi, RW, Macharia JM, Isabel N. Wagara IN, Bence RL. The medicinal properties of *Cassia fistula* L: A review.*Biomedicine and Pharmacotherapy* 144 (2021) 112240

[15] Priya, GB, Agrawal RK, Milton ARP, Mishra M, Mendiratta, SK, Kumar D Gandham RK et al.

Rapid and Visual detection of higa-toxigenic E. coli (STEC) in carabeef meat harnessing loop-mediated isothermal am[lification (LAMP). *Brazillian Journal of Microbiology*. 2024; 55: 1723-
1733.

[16]Sartorelli P, Carvalho CS, Reimao JQ, Ferreira MJP, Tempone AG.Antiparasitic activity of biochanin A, an isolated isoflavone from fruits of Cassia fistula (Leguminosae. *Parasitology Research*, 2009; 104(2), 311-314.

[17] Kushwah AS Mittal R, Kumar M, Kaur G, Prerna Goel P, Sharma RK, Kabra A and Nainwal LM. Cardioprotective Activity of *Cassia fistula* L. Bark Extract in Isoproterenol-Induced Myocardial Infarction Rat Model.Evid Based Complement Alternat Med. 2022: 6874281. 2022. doi: 10.1155/2022/6874281

[18] Pandey S, CabotPJ, Shaw, PN, Hewavitharana AK. Anti-inflammatory and immunomodulatory properties of Carica papaya. J Immunotoxicology. 2016. 13(4) : 590-602. doi: 10.3109/1547691X.2016.1149528.

[19] Sharma A, Sharma R, Sharma M, Kumar M, Barbhai MD, , Lorenzo JM et al. *Carica papaya* L.
Leaves: Deciphering Its Antioxidant Bioactives, Biological Activities, Innovative Products, and Safety Aspects.Oxid Med Cell Longev. 2022; 2022: 2451733. . doi: 10.1155/2022/2451733

[20] Mahler H. Developing protein therapeutics*Journal of Pharmacy and Pharmacology*, Volume 70, Issue 5, May 2018, Page 583, https://doi.org/10.1111/jphp.12921

[21] Singh SP, Kumar S, Tomar MS, Singh RK, Verma PK, Kumar A. Aqueous extract of *Carica papaya* leaf elicits the production of TNF-α and modulates the expression of cell Surface receptors in tumor-associated macrophages. *Biosc. Biotech Res*. 2019; 4:1115–22.

[22] Rahmani AH, Aldebasi YH. Potential role of *Carica papaya* and their active constituents in the prevention and treatment of diseases. *Int J Pharm Pharm Sci*. 2016; 8(1):11–5.

[23] Paul B, Bhuyan B, Dhar PD. Green synthesis of gold nanoparticles using *Pogestemon benghalensis*(B) O. Ktz. leaf extract and studies of their photocatalytic activity in degradation of methylene blue. *Mater Lett.https ://doi.org/10.1016/j. matlet*. 02.05414. 2013

[24] Kong YR, Jong YX, ʾBalakrishnan M, Bok ZK, Weng JKK, Tay,KC, Goh BH et al. Beneficial Role of *Carica papaya* Extracts and Phytochemicals on Oxidative Stress and Related Diseases: A Mini Review. Biology (Basel). 2021 Apr; 10(4): 287. doi: 10.3390/biology10040287

[25] Seigler DS, Pauli GF, Nahrstedt A, Leen R. Cyanogenicallosides and glucosides from Passifloraedulis and *Carica papaya*. *Phytochemistry*. 2002; 60(8):873–82.

[26] Nugroho A, Heryani H, Choi JS, Park H.J. Identification and quantification of flavonoids in *Carica papaya* leaf and peroxynitrite-scavenging activity. *Asian Pacific Journal of Tropical Biomedicine*. 2017; 7(3):208–13.

[27] Olopade OA, Henry EyinaDienye HE, Aranyo AA, Olugbojo J, Sharta, JA. Microbiological study of Sciaenid species collected from coastal waters of Niger Delta, Nigeria. Sustainable aquatic Research, 2023; 2 (3). 211-220. DOI: 10.5281/zenodo.10442400 e-ISSN: 2822-4140

[28] Elizabeth Co, Talbot, J Michaela J. Oppelt SS. Microbiology Laboratory Manual. Hayden-McNeil Publishing. ISBN: 978-073809336-9. 2017

[29] Irshad MD, Zafaryab MD, Man S., Moshahid M. 'Comparative Analysis of the Antioxidant Activity of *Cassia fistula* Extracts', *International Journal of Medicinal Chemistry*2012; Vol.12.

[30] Ghotekar S. A review on plant extract mediated biogenic synthesis of cadmium oxide nanoparticles and their recent applications. *Asian J Green Chem*2019; 3(2):187–200

[31] Adetunji CO., Olaniyi OO., Ogunkunle A.T. Bacterial activity of crude extracts of *Vernonia amygdalina* on clinical isolates. *Journal of Microbiology and Antimicrobials*. 2020; 56: 60-64

[32] Hussain A, Wahab S, Zarin I, Hussain MDS. Antibacterial activity of the leaves of Cocciniaindica (W. and A) of India. *Adv Biol Res*. 2010; 4(5): 241–248.

[33] Ahmed T, Urmi NJ, Munna MS, Das KK, Acharjee M, Rahman M.M, Noor R. Assessment of microbiological proliferation and in vitro demonstration of the antimicrobial activity of the commonly available salad vegetables within Dhaka metropolis, Bangladesh. *Am J Agri Forestr*. 2014; 2(3): 55–60.

[34]Wayne PA. CLSI. Clinical and Laboratory Standards Institute, USA. Performance standard for antimicrobial disk susceptibility tests. 14th Edition, 2024..

[35]Krishnan R, Arumugam V, Vasaviah SK. The MIC and MBC of silver nanoparticles against *Enterococcus faecalis*—a facultative anaerobe. *J. Nanomed. Nanotechnol*. 2015; 6 (2):1-4.

[36] Ajiboye EA and Olawoyin RA. Antibacterial activities and phytochemical screening of crude extract of *Carica papaya* leaf against selected pathogens. *Global Journal of Pure and*
*Applied Sciences*2020; 26: 165-170. www.globaljournalseries.com

[37]Omidiwura BRO. Qualitative and quantitative analysis of pawpaw (Carica papaya)

leaf extract and its antimicrobial effect in animal production. *Nig. J. Anim. Prod*., 2017; 44(3):78 – 83

[38]Nwofia GE, Ogimelukwe P, Eji C. Chemical composition of leaves, fruit pulp and seed in some morphotypes of Carica papaya Leaf orphotypes. *Int. J. Med. Arom. Plant*. 2012; 2:200- 206.

[39] Idhumathy J, Gurupavithra S, Ravishankar K, Jayachitra A. Green synthesis of silver nanoparticles using *Cassia fistula* leaf extract and its applications. Mintage Journal of *Pharmaceutical and Medical research*.2014; *3 (3):20-25.www.mintagejournals.com*

[40] Pawar AV, Sayali JP, Suresh GK. Uses of Cassia Fistula Linn as a Medicinal Plant. *International Journal of Advance Research and Development.* 2017; *2(3):85-91*. www.ijarnd.com