

Original Research Article

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 the *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, between November 2022-April, 2023

Methodology: The leaves were subjected to aqueous extraction, and concentrated at 50⁰C in hot air oven. The extracts were screened for biomolecules responsible for antibacterial activities.

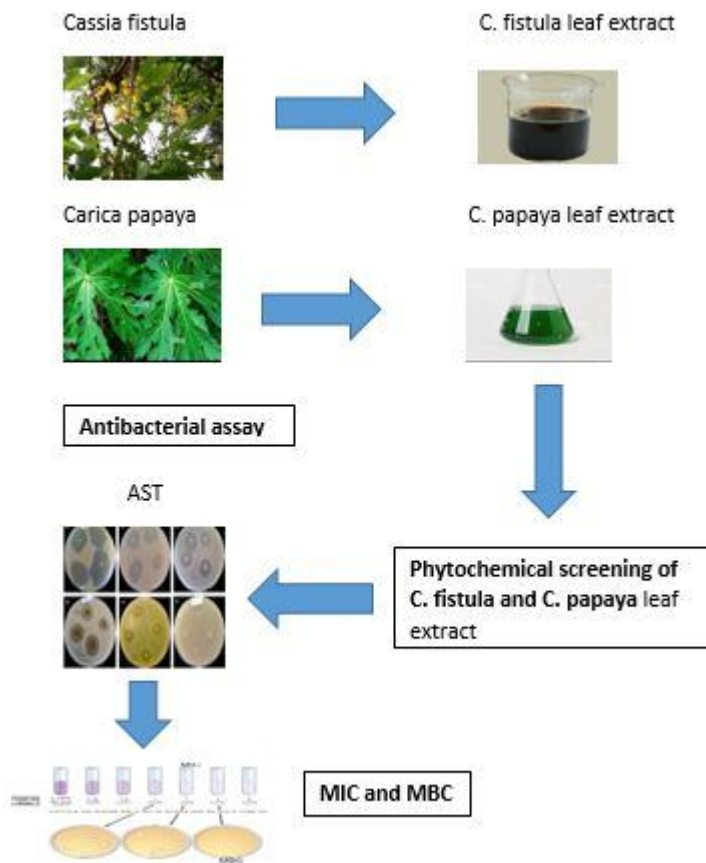
Antibacterial sensitivity test was carried out on bacteria pathogens using agar well diffusion method.

Result: The results showed that *C. fistula* leaf extract was more effective against *Aeromonas hydrophila* (20.00^b ± 2.31 mm) and *Proteus mirabilis* (17.00^b ± 1.15 mm) than *C. papaya* leaf extract. (14.00^c ± 2.31 mm and 15.00^c ± 1.15 mm), while *C. papaya* was only effective on *Vibrio 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 *Aeromonas hydrophila*,

Vibrio parahaemolyticus and *Proteus mirabilis*, except *Staphylococcus aureus* ($12.50^b \pm 1.73$ mm) and *Micrococcus 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 *Aeromonas hydrophila* than *Carica papaya* at a lesser dose with a marked difference ($P > 0.05$).

Conclusion: *Cassia fistula* leaf extract exhibited higher antibacterial efficacy than *C. papaya*, showing that it is more effective against all tested bacteria except *Vibrio parahaemolyticus*. This implies that *Cassia 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.

INTRODUCTION

Aquaculture can be defined as the farming of aquatic organisms in various aquatic environments which includes 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 presence of pathogenic bacteria in fish can limit its productivity and therefore the protein availability of the population. Gram positive and gram negative bacteria pathogens that can cause fish diseases include: *Aeromonas hydrophyla*, which causes motile Aeromonas septicemia diseases; *Vibrio Parahaemolyticus* causing acute hepatopancreatic necrosis; *Yesinia rukerica* causing enteric red-mouth diseases (Yesinosis), a chronic bacteria of intensively cultured fish; *Streptococcus* sp. such as *S. iniae* and *S. agalactiae* which are responsible for streptococcosis; *Edwasiella piscida* and *E. ictaluri* which cause enteric septicemia

of catfishes and is highly infectious; *Flavobacterium Branchiophilium*, also responsible for bacteria gill diseases (Flavibacteriosis) 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 effectiveness of medicinally valuable plants against fish pathogens. Semwal *et al* [13] reported that medicinal plants are the instant, easily available, cost-effective, efficient, eco-friendly and a highly sustainable approach to combat fish bacteria pathogens and diseases.

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, several African countries, 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 such as antidiabetic, hepato-protective, anticancer, antibacterial, wound healing, laxative, larvicidal, CNS activity, anti-itching, anti-ulcer, protease inhibitor, leukotriene inhibitors, antipyretic, anti-jussive, anti-inflammatory, antioxidant, anti-parasitic. It is also a vital herbal source for hepatic disorder and lipolipedic activity [15].

Cassia fistula Plant has a rich source of tannins, flavanoids 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, with a common name 'Pawpaw' belongs to the family: *Caricaceae*. It is native to Africa, Central America, South of Mexico, and India. It has gained several applications due to its medicinal properties. *Carica 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 effective used against gram-negative bacteria at higher doses [20]. Its seed extract contains benzyl iso-thiocyanate, which is 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 generation of radicals and thus prevent the pathogenesis [22]. Latex is one of the

most important constituents of papaya which contains papain, glycy-endopeptidase, chymopapain and caricain, and their abundance depend on different parts of papaya plant [23]

In recent years several research have been undertaken the therapeutic uses of pawpaw leaf. The leaves have been applied in the treatment of various diseases, including asthma, beriberi and jaundice [24]. Current findings shows that pawpaw leaf has several active constituents which can increase the antioxidant power in the blood and decrease lipid peroxidation level, like ascorbic acid, alpha-tocopherol, chymopapain, cyanogenic glucosides, cystatin, flavonoids, glucosinolates, and papain [25]. Various studies of the anticancer properties of papaya leaf extract (PLE) have been recorded. Many studies have been conducted on isolation and characterization of those biologically active ingredients from pawpawleaves. According to Nugroho *et al* [26], phytochemical studies shows that young pawpaw leaves contain alkaloids, saponin, tannin, flavonoid and glycosides which are responsible for its therapeutic properties [21].

The focus of this study is to evaluate and compare the antibacterial activity of *Cassia fistula* leaf extract with *Carica papaya* leaf extracts on 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 bacteria used were pathogens already isolated from *Clarias gariepinus*. The isolates include *A. hydrophila*, *V. parahaemolyticus*, *S. aureus*, *P. mirabilis* and *Micrococcus luteus*. Selective media were used to obtain pure culture of the bacteria isolates; it also facilitate

prompt growth and easy identification. *A. hydrophila*, was cultured on Ampicillin sheep blood agar (ASBA), *V. parahaemolyticus* on Thiosulphate citrate bile salt sucrose agar (TCBS), *Staphylococcus aureus* and *Micrococcus luteus* on Mannitol salt agar (MSA), *Proteus mirabilis* on Salmonella Shigella agar (SSA).

Aeromonas hydrophila was observed as haemolytic bacteria lysing the red blood cell with a clear zone (Beta haemolysis), *Staphylococcus aureus* appeared yellow on Mannitol salt agar while *micrococcus luteus* appear as small red colony, *V. parahaemolyticus* appeared green on TCBS, *Proteus mirabilis* was identified as an actively motile organism, colorless with black center. They were also subjected to microscopic examination and biochemical test for further identification. [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 *Cassia fistula* were collected from Covenant University campus while *Carica 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, Bellstech, Ota, Ogun state.

Preparation of *Cassia fistula* (Goldenshower) leaves extract

20g of the leaves were washed with deionized water, and air-dried for about two weeks. The dried *C. fistula* leaves were cut into minute sizes and boiled with 100 ml of deionized water at 70°C for 1 h. After boiling, the extract was filtered (using Whatman No. 1 filter paper), and the aqueous filtrate was then concentrated (by evaporation) at 50°C using hot-air oven, and kept in the refrigerator at 4°C for further use [29].

Preparation of *Carica papaya* (Pawpaw) leaf extract

Carica papaya (pawpaw) leaves were washed, and air-dried for about two weeks. The dried *C. papaya* leaves were cut into small pieces. 10g was weighed, washed with deionized water, and heated with 100 ml of deionized water at 70°C for 1 h. After boiling, the plant extract was filtered (using Whatman No. 1 filter paper), while the aqueous filtrates collected were concentrated (by evaporation) at 50°C using hot-air oven and kept in the fridge at 4°C for further use [21].

Phytochemical Screening of *Cassia fistula* and *Carica papaya* leaf extract

Naturally occurring biomolecules from *Cassia fistula* and *Carica papaya* leaf extract were analyzed using standard procedures according to Adetunji *et al* [30] and Ghotekaret *al* [31].

This includes: Phenols, Alkanoids, Saponins, Steroids, Flavonoids, Glycosides, Terpenoids, Proteins, and Carbohydrates.

Antimicrobial assay

Anti-bacterial activities of *Cassia fistula* and *Carica papaya* leaf extracts were conducted using agar well diffusion technique [32, 33]. The fish pathogens used include: *A. hydrophila*, *V.*

parahaemolyticus, *S. aureus*, *P. mirabilis*, and *M. luteus*. Mueller Hinton agar plates were inoculated with suspensions of the test isolates from 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, threewells 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 200mg/ml of ofloxacin(control). To allow good 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.Each sample (plant extract) was tested in two replicates, and result was recorded based on the average values.

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 [34, 35]. Muller Hinton (MHB) broth was prepared according to the manufacturer's instructions. Stock solutions containing 100mg/mL of each of the plant extract were used for the determination of the MIC. Antibacterial potencies of each of the plant extracts against five clinically isolated Gram-negative *Aeromonas. hydrophila*, *Vibrio Parahaemolyticus*, *Proteus mirabilis*, and gram positive *Staphylococcus aureus* and *Micrococcus 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 a 0.5 McFarland turbidity standard. 50 μ L 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 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 μ L) 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 the antibacterial agents (plant extract). The plates were labelled with the same code on the dilution tube where each inoculum 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 *Cassia fistula*and *Carica papaya*leafextract

Phytochemicals thatwerepresent duringthe screening carried out on*Cassiafistula*, and *Carica papaya* leaf extract are shown in Table 1.TheyincludeSaponin, flavonoids, alkaloid,betacyanin, phenols, and coumarin. 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 found in *C. fistula*. According to Table 1, other listed phytochemicals were not detected during the screening.

Table 1 Phytochemical analysisof *Cassia fistula*and *Carica papaya*leafextract

Serial Number	Phytochemicals	<i>Cassia fistula</i>	<i>Carica papaya</i>
1	Saponin	+	-
2	Flavonoids	+	+
3	Alkanoids	-	+

4	Betacyanin	+	+
5	Phenol	+	+
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 (*Aeromonas hydrophila*, *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Proteus mirabilis*, *Micrococcus luteus*) on *Cassia fistula* and *Carica papaya* leaf extract at 200mg/ml by means of inhibition zone diameters using agar well diffusion method. *C. fistula* induced higher zone of inhibition on *Aeromonas hydrophila* ($20.00^b \pm 2.31$ mm) and *Proteus 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 also more effective on *Vibrio 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 *Aeromonas hydrophila*, *Vibrio parahaemolyticus* and *Proteus mirabilis*, except *Staphylococcus aureus* ($12.50^b \pm 1.73$ mm) and *Micrococcus luteus* ($11.50^b \pm 0.58$ mm) which revealed equal inhibition zone values on *C. fistula* and *C. papaya* with no significant difference ($P > 0.05$). Ofloxacin remains significantly different ($P < 0.05$) along the

column in all tested organisms. The result also 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. <i>hydrophyla</i>	V. <i>parahaemolyticus</i>	<i>S. aureus</i>	P. <i>mirabilis</i>	<i>M. luteus</i>
<i>C. fistula</i>	20.00 ^b ± 2.31	15.50 ^c ± 0.58	12.50 ^b ± 1.73	17.00 ^b ± 1.15	11.50 ^b ± 0.58
<i>P. papaya</i>	14.00 ^c ± 2.31	17.50 ^b ± 1.73	12.50 ^b ± 1.73	15.00 ^c ± 1.15	11.50 ^b ± 0.58
Ofloxacin (control)	26.50 ^a ± 0.58	27.50 ^a ± 0.58	25.50 ^a ± 0.58	26.50 ^a ± 0.58	26.50 ^a ± 0.58

Concentration- 200 mg/ml
Control- Ofloxacin

Footnote:

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 differential alphabet: 'a', 'b, or c' along the column depicts significant difference (P<0.05)

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) recorded on *C. fistula* and *C. papaya* were shown in Table 3. The result revealed that *C. fistula* has higher potency above *C. papaya* on A.

hydrophyla ($6.25^b \pm 0.00$ 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 *C. fistula* and *C. papaya* exhibited equal inhibitory effect ($50.00^a \pm 0.00$ 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 *Aeromonas hydrophyla* which showed high significant difference ($P > 0.05$) between *C. fistula* ($20.00^b \pm 2.31$ mg/mL) and *C. papaya* ($14.00^c \pm 2.31$ mg/mL), whereas between *C. fistula* ($6.25^b \pm 0.00$ mg/mL) and Ofloxacin ($3.13^b \pm 0.00$ μ m/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 other bacteria along each column. Low MIC ($3.13^b \pm 0.00$ mg/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. hydrophyla*, and *P. mirabilis* shows 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: **Minimum Inhibitory Concentration (Mean \pm SD in mg/mL)**

Plant Extract	<i>A. hydrophyla</i>	<i>V. parahaemolyticus</i>	<i>S. aureus</i>	<i>P. mirabilis</i>	<i>M. luteus</i>
<i>C. fistula</i>	$6.25^b \pm 0.00$	$18.75^a \pm 7.21$	$50.00^a \pm 0.00$	$18.75^a \pm 7.21$	$50.00^a \pm 0.00$
<i>C. papaya</i>	$37.50^a \pm 14.43$	$12.50^a \pm 0.00$	$50.00^a \pm 0.00$	$25.00^a \pm 0.00$	$50.00^a \pm 0.00$
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

Footnote:

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

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

Minimum bactericidal concentration

Minimum bactericidal concentration of *C. fistula* and *C. papaya* were shown in Table 4. The result shows that *C. fistula* was more effective on *A. hydrophyla* ($9.38^b \pm 3.60$ mg/mL) and *P. mirabilis* ($25.00^a \pm 0.00$ mg/mL) than *C. papaya* ($50.00^a \pm 0.00$ mg/mL and $50^a \pm 14.43$ mg/mL respectively). Whereas on *V. parahaemolyticus*, *C. papaya* was more effective ($18.75^a \pm 7.22$ mg/mL) than *C. fistula* ($25.00^a \pm 0.00$ mg/mL). The result recorded when tested on *Staphylococcus aureus* and *Micrococcus 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. hydrophyla* the difference is highly significant.

Table 4 **Minimum bactericidal concentration (Mean \pm SD in mg/mL)**

Plant Extract	A.	V.	<i>S. aureus</i>	<i>P. mirabilis</i>	<i>Micrococcus</i>
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	<i>hydrophyla</i>	<i>parahaemolyticus</i>			sp
<i>C. fistula</i>	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
<i>C. papaya</i>	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

Concentration- 100 mg/ml

Control- Ofloxacin

Footnote:

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 differential alphabet: 'a', 'b', or 'c' along the column depicts significant difference (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 and effective antibacterial [13]

Result of qualitative phytochemical screening showed that biomolecules such as flavonoids, Betacyanin, phenol, and coumarins were present in both plant extracts while saponins was only found in *C. fistula*, and Alkanoid was found 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. Omidwura [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 varying degree 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. hydrophyla* 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 *Micrococcus 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 result obtained when tested *C. fistula* and Ofloxacin (control) on *A. hydrophyla* shows no significant difference, whereas on *C. papaya*, the difference is highly significant. This implies that the leaf of *Cassia fistula* has promising potentials to serve as an antibacterial agent especially against *A.*

hydrophyla 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*.

Finally, 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 mentioned on *C. fistula*, several literatures also proved that *C. fistula* leaf contains 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 *Carica papaya* leaf extract on *Aeromonas hydrophyla*, and *proteus mirabilis* while on *Staphylococcus aureus* and *Micrococcus luteus* they exhibited equal efficacy. This could be due to the fact that, *Cassia 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, Pawpaw was also seen to be more effective on *Vibrio parahaemolyticus* with no significant difference when compared with *C. fistula*.

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 an 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) *Cassia fistula* leaf extract was found to be more effective against *Aeromonas hydrophila* and *proteus mirabilis*, than *Carica papaya* leaf extract, while *C. papaya* was only more effective on *Vibrio paraheamolyticus*.
- (6) Generally, *Cassia fistula* leaf extract exhibited higher antibacterial efficacy on tested bacteria than *Carica papaya leaf extract*, and therefore recommended for the treatment of fish bacteria diseases and parasites, including multidrug resistant bacteria.

Ethical approval

No applicable

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