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Phytochemical Components and Antibacterial Activity of *Tamarindus indica* Linn. Extracts against Some Pathogens

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Authors' contributions

This work was carried out in collaboration between all authors. Author OVA designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors FEO, BOE and STO reviewed the experimental design and all drafts of the manuscript. Authors OVA and STO managed the analyses of the study. Author OVA identified the plant and performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the phytochemical composition and antimicrobial properties of tamarind extracts on some aquatic pathogenic bacteria.
Study Design: Completely Randomized Design (CRD).
Place and Duration of the Study: Department of Animal Production, Fisheries and Aquaculture, Kwara State University, Malete, Nigeria, between August 2014 and April, 2015.
Methodology: The phytochemical constituents in ordinary, warm and hot water as well as ethanol extracts of tamarind seed coat, pulp and leaves were screened. The Zone of Inhibition (ZOI)

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diameter (mm), Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against some aquatic pathogenic bacteria were determined. Data were analyzed using ANOVA at $P = .05$.

Results: The result revealed presence of reducing sugar, flavonoid, saponin and terpenoids in all tamarind extracts. The synthetic antibiotics used had significantly higher ZOI than the tamarind extracts for all the test organisms. Tamarind pulp hot water extract significantly inhibited *Aeromonas hydrophila* and *Hafnia alvei* than other extracts while the leaf warm water extracts had significantly higher zone of inhibition against *Pseudomonas putida.* The best MIC was obtained for oxytetracycline and erythromycin against *Enterobacter gergovia* and *Escherichia coli* respectively. Pulp extracts and erythromycin exhibited the same MIC, 2.56 mg/ml, for *Bacillus subtilis* and *H. alvei* while the former had lower MIC (2.56 mg/ml) against *Salmonella typhi* than the MIC (5.12 mg/ml) of the later. Oxytetracycline and tamarind extracts also demonstrated the same MIC (2.56 mg/ml) against *S. typhi*. Pulp extracts exhibited MBC for most of the test organisms.

Conclusion: Warm tamarind leaf and hot tamarind pulp aqueous extracts demonstrated better antimicrobial activities against some bacteria used in this study and hence the extracts could be used to control such microbes associated with the aquatic environment and fish products.

Keywords: Tamarind; antibacterial activity; phytochemical; minimum inhibitory concentration; synthetic antibiotic.

1. INTRODUCTION

Some of the major challenges facing fish culturists are adequate sources of low cost quality feed, availability of quality fish feed and promotion of fish health. As aquaculture becomes more and more intensive, feeds and disease prevention are significant factors in increasing the productivity and profitability of aquaculture. Hence, investing in disease prevention and treatment is crucial in aqua ventures to stay profitable [1]. Intensive aquaculture has led to growing problems with bacterial diseases and so intensive treatment with antimicrobials is required to reduce the economic losses. There are several opportunistic and pathogenic microbes that infect fish, resulting in great morbidity and mortality. Amongst such microbes are bacteria such as *Aeromonas hydrophila, Edwardsiella tarda, Flavobacterium columnare, Francisella* spp*., Pseudomonas* spp*., Mycobacterium marinum, Mycobacterium fortuinum, Streptococcus iniae* and *Staphylococcus aureus* [2].

Antibiotics at therapeutic or growth promoting levels are usually administered for short periods of time orally to sets of fish that share the same culture facility. Oxytetracycline, florfenicol, and ulfadimethoxine/ormetoprim are the antimicrobials authorized in United States of America for use in aquaculture [3]. Emerging antimicrobial resistance, due to use of antimicrobials, is a public health concern in human and animal medicine worldwide. In fish farming industry, the widespread use of the limited synthetic antibiotics for treating bacterial diseases has been associated with development of antibiotic resistance in *Aeromonas hydrophila*, *A. salmonicida, Edwardsiella tarda, E. icttaluri*, *Pseudomonas* spp., *Vibrio anguillarum, V. salmonicida, Pasteurella piscida* and *Yersinia ruckeri* [4, 5, 6]. The European Union banned their use because of the risk of chemical residues in food, the development of resistant pathogen strains which can be transferred from animals to humans, immune suppression, destabilization of helpful bacterial populations as well as the environmental pollution because up to 70-80 percent of the drug ends up in the environment [7-14].

Scientists have been searching for efficacious natural alternatives to antibiotics aimed at promoting animal health. Such alternatives include phytobiotics, probiotics, synbiotics and organic acid. The antimicrobial activities of phytobiotics (phytogenics) such as tamarind [15, 16, 17], black pepper, curry leaf, coriander [18] turmeric and ginger [19], onion and walnut leaf [20], essential oil from Pakistani spices [21] and leaves, bark and root of guava among others [22] have been investigated as possible alternatives to the synthetic antibiotics. The antibacterial activities from plant origin have been linked to the presence of bioactive phytochemicals in such plants. Phytochemicals contain secondary metabolites such as alkaloid, saponin, tannin, terpenoids and phenolic compounds which have been associated with antimicrobial, antioxidants and anti-inflammatory properties [23,24].

Tamarindus indica Linn. (tamarind), a multipurpose tree widely available in the tropics, is of great importance in traditional medicine. The leaves and bark of the plants have been utilized for the treatment of body pain, yellow fever and stomach disorders traditionally [15]. Compounds such as carvacrol, cinnamaldehyde, epicathechin, lupeol, tartaric acid are components of tamarind [25, 26, and 27]. [28 and 29] also reported antibacterial, antifungal, antiviral, antioxidant, carminative, digestive and laxatives activities of tamarind. Most of the earlier researchers on the use of natural alternatives to antibiotics had focused mainly on pathogens relating to human and terrestrial live stocks. Therefore, the aim of this study was to determine the phytochemical composition and antimicrobial properties of tamarind extracts against some aquatic pathogenic organisms.

2. MATERIALS AND METHODS

2.1 Source of Plant Materials and Preparation

Tamarind leaf and fruit were obtained from the environment of Teaching and Research Farm College of Agriculture, Kwara State, University, Malete. The plant parts were taken to the herbarium of the Department of Botany, University of Ibadan and the plant was identified as *Tamarindus indica* Linn. and given the Voucher Number: UIH-22550. The fruit husk was carefully removed, the pulp was scrapped from the seeds, remnant of pulp was washed and the seed coat removed. The leaves, pulp and seed coats were air-dried under shade.

2.2 Plant Extraction

Both the leaves and seed coats of tamarind were ground with blender, while the pulp was blend with small volume of the solvent for extraction and later top up to the required volume. The extraction of tamarind leaf, seed coat and pulp was carried out using maceration method with distilled water and ethanol. Each sample was mixed with ordinary distilled water, warm distilled water at 50°C, Hot distilled water at 80°C [30] and 96% ethanol at a ratio 1:10 (w/v) [31]. The mixtures of plant parts were homogenized and kept on rotary shaker [32] for 2 days. The homogenized mixtures were centrifuged (SE-CF-TDZ-WS, Labkits, U-Therm International (Hong Kong) Limited) at 4000 rpm for 30 minutes at room temperature and the supernatant collected, sieved with double layer of muslin cloth after which it was filtered through Whatman No.4 filter paper. The solvents were removed under vacuum using a rotary evaporator (IKA® RV10, Artisan Technology Group, Champaign, US) at 60°C for ethanol and 90°C for water. The concentrated extracts were further dried in freeze-drier (LYOTRAP, LTE Scientific Ltd., Great Britain) and kept in freezer before use.

2.3 Qualitative Phytochemical Screening of Tamarind Extracts

The extract of the seed coat, the pulp and the leaves of tamarind were evaluated for qualitative determination of major phytoconstituents which include reducing sugar, terpenoids, alkaloids, cardiac glycosides, flavonoids, saponins and tannins as described by Sofowora [33] and Trease and Evans [34].

2.4 *In vitro* Screening of Antimicrobial Activity of Tamarind Extracts

2.4.1 Source of microorganisms

Pure isolates of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Pseudomonas putida*, *Enterobacter gergovia*, *Hafnia alvei* and *Aeromonas hydrophila* were obtained from the laboratory stock of the departments of Microbiology and Veterinary Medicine, University of Ibadan, Nigeria. The organisms were sub-cultured on nutrient agar in plates within 24 hrs at 37°C and thereafter the isolates were grown on nutrient agar slants and preserved in refrigerator at 4°C during the study.

2.4.2 Agar well diffusion assay

The antimicrobial activity of aqueous and ethanolic extracts of tamarind leaf, seed coat and pulp against the aforementioned isolates was determined using standard methods [35,36]. The bacteria were sub-cultured from the preserved slants for 24 hour before use. Mueller-Hinton Agar was prepared, sterilized, allowed to cool to room temperature and then poured into plates to about 4mm depth under an aseptic condition. 24 hour old culture of each test organisms was standardized to 0.5 McFarland standards (10^6 CFU/ml). About 100 µl of the standardized cell suspensions was spread on Mueller-Hinton agar plates in triplicates. Four wells were bored on each plate with a sterile 6mm diameter cork borer; 100 µl of the crude extracts at 10 mg/ml were introduced into the wells, allowed to stand at room temperature for about 30 minutes. Controls were set up in parallel using the solvent used for extraction as well as two synthetic

antibiotics, oxytetracycline and erythromycin commonly used in aquaculture and livestock industry as therapeutic agents. The volume and concentration of the synthetic antibiotics were the same with those of the tamarind extracts. The plates were incubated at 37°C for 24 h and then observed for inhibition zone diameter (mm).

2.4.3 Minimum inhibitory concentration of tamarind extracts

Estimation of Minimum Inhibitory Concentration (MIC) of the tamarind extracts was carried out using agar dilution method. Two-fold dilutions of antimicrobial agents were prepared as described by CLSI [36] from 10.24 mg/ml using distilled water as diluents. Briefly, 18mls Mueller Hinton Agar (MHA) was prepared in McCartney bottles and sterilized. The sterilize MHA was allowed to cool to 50°C in water bath after which 2 mls of each diluted antimicrobial agent was gently mixed with MHA and poured into sterilized petri-dishes under aseptic condition. This was allowed to gel and cooled for 1 hour. A 24-h old culture of each of the test organisms was serially diluted in 0.85% sterilized saline water to standardize the organisms to 0.5 McFarland standards (10^6 CFU/ml). 1ml syringe was used to deliver 2 drops of the standardized inoculums to 100 mm diameter plate equivalent to approximately 40 µl per plate. The inoculum was spread on the agar surface and the plates were allowed to stand at room temperature for about 30 minutes to ensure the moisture in the inoculum is absorbed into the agar. The plates were then inverted and incubated at 37°C. The plates were thereafter observed after 20 to 24-hour incubation period for growth of organism. The lowest concentration of tamarind extracts and the synthetic antibiotics that completely inhibits growth of the inoculum was recorded as MIC.

2.4.4 Minimum bactericidal concentration (MBC) of tamarind extract

Sterile inoculating loop was used to pick from the MIC plates and streak on a sterilized MHA plate surfaces. The inoculated plates were incubated at 37°C for 24hour. The lowest concentration in which tamarind extracts and the synthetic antibiotics did not allow growth of organisms on the MHA plates was recorded as MBC.

2.5 Statistical Analysis

One-way Analysis of Variance (ANOVA) was used to analyze the data on zones of inhibition. Duncan multiple range tests was used to compare differences among means at 5% probability level using statistical software SAS (Statistical Analysis System, 2010).

3. RESULTS AND DISCUSSION

3.1 Phytochemical Constituents in Tamarind Extracts

The result on phytochemical screening of tamarind extracts (Table 1) revealed presence of reducing sugar, flavonoid, saponin, terpenoids while tannin and cardiac glycosides were absent.

Table 1. Results of qualitative phytochemical screening of tamarind extract

Samples	Phytochemicals						
	Alkaloid	Cardiac glycosides	Flavonoids	Reducing sugar	Saponin	Tannin	Terpenoid
LOW	+	-	+	+	+	-	+
LWW	+	-	+	+	+	-	+
LHW	+	-	+	+	+	-	+
LET	+	-	+	+	+	-	+
POW	+	-	+	+	+	-	+
PWW	+	-	+	+	+	-	+
PHW	+	-	+	+	+	-	+
PET	+	-	+	+	+	-	+
SOW	+	-	+	+	+	-	+
SWW	+	-	+	+	+	-	+
SHW	+	-	+	+	+	-	+
SET	+	-	+	+	+	-	+

LOW = Leaf Ordinary Water; LWW = Leaf Warm Water; LHW = Leaf Hot Water; LET = Leaf Ethanol; POW = Pulp Ordinary Water; PWW = Pulp Warm Water; PHW = Pulp Hot Water; PET = Pulp Ethanol; SOW = Seed Coat Ordinary Water; SWW = Seed Coat Warm Water; SHW = Seed Coat Hot Water; SCET = Seed Coat Ethanol

3.2 Antimicrobial Activities of Tamarind Extracts

3.2.1 Zones of inhibition of tamarind extracts

Table 2 shows the results of zone of inhibition of the tamarind extracts compared to the synthetic antibiotics. The synthetic antibiotics used had significantly higher (P = .05) zones of inhibition than the tamarind extracts for all the test organisms. Tamarind Pulp Hot Water (PHW) extract significantly inhibited *Aeromonas hydrophila* better than other extracts while Leaf Ethanolic (LET) extract had the lowest zone of inhibition against *A. hydrophila*. Leaf Warm Water (LWW) extracts had significantly higher (P = .05) zone of inhibition against *Pseudomonas putida*. Higher significant zone of inhibition was also exhibited by PHW extract against *Hafnia alvei*. The zones of inhibition of LWW, LHW, PHW extracts against *Escherichia coli* were significantly higher (P = .05) than other extracts while the seed coat showed no antimicrobial activities against *E. coli* and *Bacillus subtilis*. Pulp Ethanol Extract (PET) had significantly higher (P = .05) inhibition (12.00mm) against *Salmonella typhi*.

3.2.2 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of tamarind extract

MIC and MBC of aqueous and ethanolic extracts of tamarind against the test organisms in comparison to erythromycin and oxytetracycline are shown in Table 3. The lowest MIC, 0.64mg/ml, was obtained for oxytetracycline and erythromycin against *E. gergovia* and *E. coli* respectively. Amongst the leaf extracts, LWW extract exhibited lower MIC against all the test organisms except for *P. putida* and *S. typhi* against which higher values were obtained. Similar value of MIC, 2.56 mg/ml, was also exhibited by all tamarind pulp extracts against the test organisms except for *P. putida* while higher values of MIC were obtained from seed coat extracts. MBC value of 1.28 mg/ml was obtained for oxytetracycline against *A. hydrophila* and MIC of 2.56 against *S. typhi*. MBC value of 2.56 mg/ml was similarly exhibited by POW and PWW extracts against *S. typhi* as well as against *E. coli* and *S. aureus* by PHW and PET extracts: MBC was not exhibited by the synthetic antibiotics against these three pathogens.

Table 2. Antagonistic activity (mm) of synthetic antibiotics and tamarind extracts at 10mg/ml against some pathogens

TE/A	Pathogens							
	A. hydrophila	***P. putida***	***E. gergovia***	***H. alvei***	***E. coli***	***S. aureus***	***B. subtilis***	***S. typhi***
Solvents	0.00^{f}	0.00^{h}	0.00^{i}	0.00^{k}	0.00^{f}	0.00^{c}	0.00^{g}	0.00^{h}
ERY	21.33^{a}	22.00^{a}	26.00^{a}	15.00^{b}	32.67^{a}	28.33^{a}	26.67^{a}	25.67^{b}
OTC	22.33^{a}	13.33^{b}	15.00^{b}	21.00^{a}	25.67^{b}	27.67^{a}	22.00^{b}	26.33^{a}
LOW	9.67^{de}	9.33^{efg}	9.67^{de}	11.00^{g}	9.33^{e}	9.67^{b}	10.00^{c}	10.00^{e}
LWW	11.67^{c}	12.00^{c}	11.00^{c}	11.67^{e}	10.67^{cd}	11.00^{b}	11.00^{c}	10.00^{e}
LHW	10.67^{cd}	10.00^{de}	11.00^{c}	11.00^{g}	$11.00^{c.}$	11.00^{b}	11.67^{c}	11.00^{d}
LET	8.67^{e}	8.67^{fg}	9.33^{g}	10.00^{j}	9.00^{e}	9.00^{b}	9.00^{f}	9.00^{g}
POW	10.33^{cde}	9.67^{def}	10.00^{e}	11.33^{f}	9.00^{e}	9.00^{b}	9.67^{ef}	10.00^{e}
PWW	10.67^{cd}	10.00^{de}	10.00^{d}	11.67^{e}	9.33^{e}	9.00^{b}	9.67^{c}	11.00^{d}
PHW	13.33^{b}	10.67^{d}	11.00^{c}	13.33^{c}	11.33^{c}	9.00^{b}	9.33^{c}	11.00^{d}
PET	9.67^{de}	9.00^{efg}	10.00^{d}	12.00^{d}	9.67^{e}	9.00^{b}	9.00^{c}	12.00^{c}
SOW	9.33^{de}	8.33^{g}	9.00^{f}	10.00^{j}	0.00^{f}	9.00^{b}	0.00^{e}	9.67^{f}
SWW	10.33^{cde}	9.00^{efg}	9.00^{f}	10.67^{h}	0.00^{f}	9.00^{b}	0.00^{e}	9.67^{f}
SHW	11.00^{cd}	9.67^{def}	10.00^{d}	10.33^{i}	0.00^{f}	9.00^{b}	6.00^{d}	10.00^{e}
SET	11.00^{cd}	9.00^{efg}	11.33^{c}	10.00^{j}	0.00^{f}	9.00^{b}	0.00^{e}	9.00^{g}
SEM	0.578	0.423	0.158	0.274	0.428	0.765	0.942	0.318

Means with the same letter on the same column are not significantly different at P = .05

TE/A = Tamarind extracts/Antibiotics ERY= Erythromycin OTC = Oxytetracycline LOW = Leaf Ordinary Water LWW = Leaf Warm Water LHW = Leaf Hot Water LET = Leaf Ethanol POW = Pulp Ordinary Water PWW = Pulp Warm Water PHW = Pulp Hot Water PET = Pulp Ethanol SOW = Seed Coat Ordinary Water SWW = Seed Coat Warm Water SHW = Seed Coat Hot Water SET = Seed Coat Ethanol A = Aeromonas P = Pseudomonas E = Enterobacter H = Hafnia E = Escherichia S = Staphylococcus B = Bacillus S = Salmonella

Table 3. Minimum inhibitory concentration and minimum bactericidal concentration (mg/ml) of two synthetic antibiotics and tamarind extracts against some pathogens

TE/A	Pathogens							
	A. hydrophila	***P. putida***	***E. gergovia***	***H. alvei***	***E. coli***	***S. aureus***	***B. subtilis***	***S. typhi***
ERY	1.28	1.28	1.28	2.56	0.64	1.28	2.56	5.12
OTC	1.28*	2.56	0.64	1.28	1.28	1.28	1.28	2.56
LOW	10.24	10.24	5.12	5.12	5.12	5.12	5.12	5.12
LWW	2.56	10.24	2.56	2.56	2.56	2.56	2.56	5.12*
LHW	5.12	5.12	2.56	2.56	2.56	5.12	5.12	5.12*
LET	2.56	5.12	2.56	2.56	5.12	10.24	5.12	5.12*
POW	2.56	5.12	2.56	2.56	2.56	2.56	2.56	2.56*
PWW	2.56*	5.12	2.56	2.56	2.56	2.56	2.56*	2.56*
PHW	2.56	5.12	2.56*	2.56	2.56*	2.56*	2.56	2.56
PET	2.56	5.12	2.56	2.56	2.56*	2.56*	2.56	2.56
SOW	10.24	10.24	5.12	10.24	10.24	NA	10.24	NA
SWW	10.24	10.24	5.12	5.12	5.12	5.12	5.12	5.12
SHW	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12
SET	10.24	10.24	10.24	10.24	10.24	NA	10.24	10.24*

**Minimum Bactericidal Concentration NA- Not Active at the highest concentration used TE/A = Tamarind extracts/Antibiotics ERY= Erythromycin OTC = Oxytetracycline LOW = Leaf Ordinary Water LWW = Leaf Warm Water LHW = Leaf Hot Water LET = Leaf Ethanol POW = Pulp Ordinary Water PWW = Pulp Warm Water PHW = Pulp Hot Water PET = Pulp Ethanol SOW = Seed Coat Ordinary Water SWW = Seed Coat Warm Water SHW = Seed Coat Hot Water SET = Seed Coat Ethanol A = Aeromonas P = Pseudomonas E = Enterobacter H = Hafnia E = Escherichia S = Staphylococcus B = Bacillus S = Salmonella*

3.3 Discussion

This result of the phytoconstituents of tamarind in this study is similar to what has been reported by other researchers [15, 16, 37] on the phytoconstituents of tamarind. However, the absent of tannins from this study is contrary to other reports. Antibacterial activity obtained from tamarind extracts in this study coincided with the reports of other researchers on antibacterial activities of phytogenics. [15] similarly reported higher zones of inhibition from synthetic antibiotic compared with tamarind extracts; however higher zone of inhibition and lower MIC values were obtained from leaf extract in this study. Also in contrast to Dhoughari [15], not all extracts in this study exhibit bactericidal (MBC) activity [16] also reported better antibacterial activity of aqueous tamarind pulp extract compared to the ethanolic extract against *P. aeruginosa*. Furthermore, lack of clear or lower zone of inhibition discovered in this study against *S. typhi* and *S. aureus* coincided with earlier observation [16].

Higher zones of inhibition obtained from oxytetracycline compared to tamarind extracts against the test organisms is in agreement with the report of [17] but lower MIC values were obtained from the tamarind aqueous extracts against *A. hydrophila* and *S. aureus* than what the researchers reported from clove aqueous extract. Contrary to the absence of antimicrobial activity reported [19] on turmeric and ginger root aqueous extract, tamarind pulp and leaf aqueous extracts in our study exhibited antibacterial activities against *Pseudomonas* and *E. coli*. The absence of antibacterial activities of the seed coat extracts against *E. coli* and *B. subtilis* is similar to the observation of [20] on lack of antibacterial activities of onion bulb and walnut leaf extracts against *B. subtilis* and *E. coli* respectively.

Lower MIC values were discovered in this study from all tamarind pulp extracts as well as warm and hot aqueous leaf extracts than the MIC values reported [21] on Pakistani spices against *E. coli* and *S aureus*. The MIC value of the plant extracts examined [22] against *E. coli* is similar to 2.56 mg/ml obtained in this study while lower value was obtained in this study against *A. hydrophila* than what the authors reported. Generally, the isolates investigated in this study were more sensitive to warm and hot water extracts than to ordinary water extracts and ethanolic extracts. The higher antibacterial activity of the warm and hot aqueous extracts in this study might be an indication of higher solubility of phytoconstituents in water at higher temperature than lower temperature. The demonstration of better antibacterial activity from warm and hot aqueous extract provides the scientific basis for the boiling of herbs by the traditional folks in disease treatments. The use of

the aqueous extracts is of better economic advantage for fish farmer because ethanol is more costly than distilled water.

4. CONCLUSION

The antibacterial activity demonstrated by tamarind extracts in this study shows that the extracts could be used to control bacterial associated with the aquatic environment and fish products. The discovery from this study is an indication that tamarind pulp and leaf warm/hot extract could be used as possible phytogenic to control *Aeromonas* and *Pseudomonas* infection in fish as well as protect fish products from poisoning organisms. Further study is however needed on the concentration of tamarind extracts that would be as effective as synthetic antibiotics, *in vivo* toxicological investigation and performance of farmed fish using warm and hot aqueous extracts of tamarind leaf and pulp.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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