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COMPARATIVE ACTIVITIES OF PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF LEAF EXTRACTS OF *Bryophyllum pinnatum* (Lam.), *Alchornea cordifolia* (Schumach. & Thonn.), *Acalypha wilkesiana* (Muell. Arg) AND SEED EXTRACT OF *Citrullus lanatus* (Thunb.)

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Abstract

The comparative activities of the phytochemical constituents and antioxidant properties of leaf extracts of *Bryophyllum pinnattum, Alchornea cordifolia* and *Acalypha wilkesiana,* and seed extract of *Citrullus lanatus* with their antimicrobial properties were investigated. Qualitative and quantitative phytochemical profile, antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) of the obtained ethanolic extract were analysed. Using an agar dilution assay, the antimicrobial effects of the ethanolic extracts on the typed *Streptococcus faecalis and Pseudomonas aeruginosa* were determined. All of the studied extracts contained saponins, tannins, phenols, flavonoids, steroids, terpenoids, reducing sugars, alkaloids and cardiac glycosides; alkaloids was however absent in *Citrullus lanatus* seed extract. Overall DPPH radical scavenging and FRAP activities of the sample extracts showed a dose-dependent response range of 17.50±0.50 - 89.50±0.50 µg/ml and 0.254±0.002 - 0.434±0.004 µg/ml respectively. *Streptococcus faecalis* and *Pseudomonas aeruginosa* showed significant susceptibility to *A. wilkesiana and A. cordifolia* leaf extract at 400mg/ml compared to less susceptibility at lower concentrations. *A. cordifolia* leaf extract at MIC 0.16 mg/ml showed highest inhibition to *S. faecalis* whereas the least inhibition was observed in *A. wilkesiana* leaf extract against *P. aeruginosa* at MIC of 2.56 mg/ml. Extracts of *A. cordifolia* and *A. wilkesiana* contains phytochemical compounds with significant antioxidant and antimicrobial properties and could be harnessed as natural antioxidant and antibiotics.

Keywords: **phytochemistry, antioxidant activity, antimicrobial activities, *Bryophyllum pinnatum, Alchornea cordifolia, Acalypha wilkesiana, Citrullus lanatus*.**

1. INTRODUCTION

Aquaculture has expanded dramatically over the past three decades and with that growth has come the introduction or re-appearance of a variety of infectious diseases [1]. High-density fish populations generally associated with intensive aquaculture create excellent conditions for the introduction and spread of infections, resulting in serious monetary losses. Typically, pathogen eradication, antibiotic therapy, prophylactic measures including the use of probiotics, or immunizations are used to prevent or control prevalent infectious diseases [2]. Particularly in situations where antibiotics are misused, the traditional approaches to disease management are vulnerable to the development of resistant bacteria. The recent appearance of bacterial strains with reduced susceptibility to antibiotics and the rising prevalence of multidrug resistant bacteria strains create severe concerns for delivering expected levels of wholesome health management in aquaculture production systems [3]. *Aeromonas salmonicida, Edwardsiella tarda, Mycobacterium peregrinum, Salmonella sp., Flavobacterium columnare* are common fish pathogens with recorded high resistance rate to tetracycline, streptomycin, and kanamycin [4, 5], among many others.

Pseudomonas aeruginosa was reported to be highly invasive and cytotoxic pathogen which is resistant to tetracycline, ampicillin, and erythromycin in infected wild and cultured populations of *Sparus aurata* [6]. Similar high resistance was observed in *Staphylococcus aureus* and *Streptococcus dysgalactiae* to tetracycline and macrolide therapies [7,8]. It has been shown that bacteria isolated from human and animal diseases, including aquaculture, are continuously exchanging resistance genes [9]. The epidemiological and genetic evidence further affirmed the spread and transmission of resistance genes from fish pathogens such as *Aeromonas* sp. to human pathogens such as *Escherichia coli*. The excessive use of antibiotics in industrial aquaculture have led to increased

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levels of residual antibiotics in fish and shellfish which in turn is raising the resistance rates in cultured species [10,11].

Based on the presence of various antioxidants and antibacterial phyto-constituents, several publications have demonstrated that medicinal plant extracts are potential sources of natural antioxidant and antimicrobial agents [12,13]. Screening medicinal plants for active chemicals such as flavonoids, pigments, phenols, terpenoids, steroids, alkaloids, and essential oils [14] has become more crucial as possible sources of natural antibiotics due to the increased adoption of herbal medicine as an alternative to conventional disease management [15]. Plant such as *Bryophyllum pinnatum* (also known as, *Kalanchoe pinnata*) [16], *Alchornea cordifolia*, *Acalypha wilkesiana* [17,18], and *Citrullus lanatus (*Watermelon) [19,20] are applied for the cure of infectious disorders linked with ear ache, burns, abscesses, ulcers, insect bites, whitlow, diarrhoea and lithiasis [21]. Extracts from these plants leaves and seed have been used as a traditional remedy for a variety of ailments, especially in humans, for decades; as well as sources of natural antioxidant, which could make them relevant in the management of cultured fish diseases [22]. Therefore, the study aimed to investigate the comparative activities of the phytochemical constituents, antioxidant and antimicrobial properties of leaf extracts of *Bryophyllum pinnatum*, *Alchornea cordifolia* and *Acalypha wilkesiana*, and seed extract of *Citrullus lanatus.*

2. MATERIALS AND METHODS

The Assay Organisms

The pathogenic strains of *Streptococcus faecalis* and *Pseudomonas aeruginosa* used in the study were received from the Pharmaceutical Microbiology Laboratory of the Department of Pharmaceutics and Pharrmaceutical Technology, Faculty of Science, University of Lagos, Nigeria. These isolates are peculiar pathogenic strains showing similar pathogenicity and antimicrobial resistance pattern with infected fish samples. *Pseudomonas aeruginosa* was grown on Pseudomonas Cetrimide Agar (PCA) and *Streptococcus faecalis* on salted MacConkey Agar (MAC) containing 6.5% sodium chloride to suppress other contaminants. The isolates were sub-cultured and maintained on Mueller Hinton Agar at 37ºC.

Collection of leaves and extract preparation: Fresh leaves of *Bryophyllum pinnatum* (Lam.), *Alchornea cordifolia* (Schumach. & Thonn.) and *Acalypha wilkesiana* (Muell. Arg) were sourced from the Botanical garden, University of Lagos and verified by Dr. A.B. Kadiri of Botany Department, University of Lagos. Dried seeds of *Citrullus lanatus* (Thunb.) were procured from a local market in Lagos state, Nigeria. The sampled leaves and seeds were thoroughly cleaned with water and allowed to air dry at room temperature (25±2°C). Following the protocol of Azlim-Almey *et al* [23] with slight modification, the dried leaves were ground into powder and extracted using ethanol. The dried samples were transferred separately into beakers and 400ml of 70% ethanol was added respectively. Each beaker was shaken vigorously (200 rpm) on an orbital shaker (CO-Z) for 24 hours; the extract was separated from the sample through filtration (Whatman No.1), concentrated with rotary evaporator (RE-52A, Shangai, China) and stored at 4ºC prior to use.

Phytochemical assay

The phytochemical constituents of the extracts were determined using the standard methods of Sofowora [24] and Trease and Evans [25]. Thin-layer paper chromatographic techniques were used to separate the extracts' components for proper identification. For alkaloids detection, the extracts were dissolved separately in 1% aqueous hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids using Dragendroffs test. Formation of red precipitate indicated the presence of alkaloids. Flavonoids were detected using lead acetate test while Steroids were analysed by adding 2 ml acetic anhydride to 5 mg of the extracts each with 2 ml of H_2SO_4. A shift in color from violet to blue or green shows the presence of steroids. Salkowski's test was used to identify terpenoids, which were found by looking for an inner face with a reddish-brown color after 5 mg of the leaf and seed extracts were combined with 2 ml of chloroform and 3 ml of concentrated H_2SO_4. Phenols were detected using ferric chloride test using 5ml extracts treated with few drops of ferric chloride solution. Blueish black coloration formed suggested the presence of phenols. Saponins were detected from the presence of froth formed when the plant extracts were shaken in distilled water. Similarly, dark green colouration formed as a result of addition of $FeCl_3$ to filtrate derived from the heated mixture of water and extracts was used to detect tannins. Benedict's test was used to determine the presence of reducing sugar while cardiac glycoside followed Keller-Killani test.

Quantitative Phytochemical metabolites

Essential secondary metabolites were quantified in all the extracts.

Alkaloids: Total alkaloid was determined following the spectrophotometric method described by Shamsa *et al* **[26]** The absorbance of the complex formed from the mixture of the prepared extract, 5 ml of bromocresol green solution and 5 ml of phosphate buffer was read at 470 nm against the reagent blank using a UV-visible spectrophotometer (ThermoSpectronic, Biomate 3, USA). Atrophine was used as standard and the amount of atropine equivalents (mg of AE/g of extract) represented the total alkaloid content.

Steroids: The method described by Balogun *et al* [27] was slightly modified and used to assay the total steroids of the plant extracts. Briefly, 4N H_2SO_4, 0.5%$FeCl_3$ and 0.5% $C_6N_6FeK_3$ were added to 1ml of the test extract. After heating for half an hour the mixture was diluted with distilled water and the absorbance was read at 780 nm against a reagent blank. The total amount of steroids was expressed as cholesterol equivalents (mg of CHO/g of extract).

Flavonoids: The total flavonoid concentration was determined by the aluminium chloride spectrophotometric test using quercetin (20, 40, 60, 80, and 100 g/ml) to prepare standard calibration curve. The total flavonoid content was expressed in

terms of quercetin equivalents (mg of QE/g of extract) [28,29,30].

Phenols: The determination of the total phenol content followed the Folin-Ciocalteu procedure of Singleton *et al* [31]. Gallic acid standard as well as test solutions were prepared using Folin-Ciocalteu reagent and Na_2CO_3. Their optical densities were read at 550nm against the reagent blank, expressing the total phenol content as gallic acid equivalent (mg of GAE/g of extract).

Tannins: The determination of tannins followed similar protocol to that of total phenol using the Folin-Ciocalteu procedure of Singleton *et al* [31]. However, the absorbance of the test and the gallic acid standard solutions were read at 725 nm and the tannin contents of the plant extracts were expressed as gallic acid equivalent (mg of GAE/g of extract).

Reducing Sugars: The 3,5-dinitrosalicyclic (DNS) acid protocols of Iqbal *et al* [32] and Chai et al [33] were used for reducing sugar assay. Dinitrosalicyclic reagent was prepared following standard procedure with the use of dinitrosalicylic acid, NaOH and sodium potassium tartrate and kept in darkness at room temperature. The reagent was added to 1ml of the extract and boiled for 10 minutes. Glucose standard (100mg/ml) was similarly arranged and the optical density of the test and standard solutions were measured at 540nm. The reducing sugar content was expressed glucose standard equivalence.

Antioxidant Assay

DPPH radical scavenging assay: The spectrophotometric analysis of Kulkarni e*t al* [34] was used to determine 2,2-diphenyl-1-picrylhydrazyl radical scavenging properties of the extract. 1 ml of extract was mixed with 1 ml of a 0.1 mM solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol at various doses of 25 to 100 g/ml. After giving the combination a thorough shake, the mixture was let to stand at room temperature for 30 minutes. A UV-visible spectrophotometer was then used to detect the absorbance at 517 nm.

Ferric reducing antioxidant power assay (FRAP): The slightly modified method of Oyaizu [35] was used in assaying the reducing power of the extracts. 0.1 M sodium phosphate buffer (pH 6.6), 2.5 ml of sample extract produced at various concentrations (25–100 g/ml), and 2.5 ml of 1% K3Fe(CN)6 were combined and incubated at 50°C for 20 minutes. After adding 2.5 ml of trichloroacetic acid (10%, w/v), the mixture was spun at 5000 rpm for 10 minutes. The absorbance at 700 nm was measured in comparison to a blank after the upper layer (5 ml) had been combined with 0.5 ml of freshly prepared FeCl3 (0.1%, w/v). The control substance utilized was gallic acid.

Antimicrobial Activity: Agar well dilution assay was used as described by Hugo and Russel [36]. Dropwise additions of the bacterial liquid cultures were made to the normal saline solution until the turbidity of the mixture reached the densitometer's 0.5 McFarland turbidity standard. To each well bored to 10mm diameter on Mueller Hinton agar plate already seeded with adjusted 0.5 McFarland turbid test organism, 150 µl of graded diluted extracts of 0.0025 to 10.24 mg/ml was added and allowed to diffuse. The plates underwent a 24-hour incubation at 37^{o}C before being examined for zones of inhibition brought on by bacterial growth. Zone reader readings were taken multiple times, and average zone values were calculated and noted.

Data analysis: The experimental data obtained were expressed as Mean ± SE and the significance of the phytochemicals and antioxidant activities were determined using one-way Analysis of Variance (ANOVA) as described by Morales-Luna et al [37]. Using Duncan's Multiple Range Test, differences among means were evaluated for significance at 95% confidence level.

3. RESULTS

Preliminary phytochemical detection:

All of the studied extracts contained saponins, tannins, phenols, flavonoids, steroids, terpenoids, reducing sugars, alkaloids and cardiac glycosides, with the exception of *C. lanatus* seed extract, which lacks alkaloids (Table 1).

A. cordifolia (43.24±0.40 mg/100g) contained significantly more saponin relative to other extracts. Tannin and phenol in *A. wilkesiana* and *A. cordifolia* did not vary substantially ($P>0.05$); their values, however, were significantly ($P<0.05$) higher than those in *B. pinnatum* and *C. lanatus*. *Citrullus lanatus* had significantly ($P<0.05$) more flavonoids (78.36±0.16 mg/100g) and steroid (78.99±0.21 mg/100g) compared to other extracts. The levels of cardiac glycoside which ranged from 32.76±0.33 mg/100g in *C. lanatus* to 37.59±0.25 mg/100g in *A.cordifolia* varied substantially ($P<0.05$) across sample extracts. Comparatively higher ($P<0.05$) reducing sugar (69.94±0.14 mg/100g) was observed in *B. pinnatum* leaf extract (Table 2).

Antioxidant activities: The scavenging capacity (DPPH Free Radical Scavenging) of the sample extracts showed a dose-dependent response and ranged from 17.50±0.50 to 89.50±0.50 across all concentrations. Ascorbic acid, used as the standard, showed significantly higher activities across all selected concentrations relative to the extracts. *A. wilkesiana* showed significantly higher activities relative to the three other sample extracts across all concentrations ($P<0.05$). At 50 µg/ml concentration, the radical scavenging activity of *C. lanatus* (40.50±0.50), *A. cordifolia* (38.50±0.50) and *B. pinnatum* (37.50±0.50) were similar ($P>0.05$, Fig 1). Similarly, there was increase in Ferric Reducing Antioxidant Power (FRAP) values with increasing concentrations of the sample extracts with ascorbic acid showing significantly ($P<0.05$) higher activities across all selected concentrations relative to the extracts (Fig 2). *B. pinnatum* showed significantly higher FRAP activity at concentration of 25 µg/ml (0.254±0.002), 50 µg/ml (0.291±0.002) and 75 µg/ml(0.352±0.002) relative to the other leaf extracts. At 100 µg/ml concentration, *A. wilkesiana* (71.50±0.50) and *C. lanatus* (0.434±0.004) exhibited significantly ($P<0.05$) more FRAP activity relative to those of *A. cordifolia* and *B. pinnatum.* Comparatively more ($P<0.05$) total antioxidant

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Table 1: Qualitative analysis of phytochemical compounds

Plant extract	Saponin	Tannin	Phenol	Flavonoid	Steroid	Terpenoid	Reducing sugar	Alkaloid	Cardiac glycoside
B. pinnatum	+	+	+	+	+	+	+	+	+
A.cordifolia	+	+	+	+	+	+	+	+	+
A.wilkesiana	+	+	+	+	+	+	+	+	+
C. lanatus	+	+	+	+	+	+	+	-	+

Table 2: Quantitative Phytochemical profile of the plant extracts

Plant extract	**Quantitative (mg/100g)**							
	Saponin	Tannin	Phenol	Flavonoid	Steroid	Cardiac glycoside	Reducing sugar	Alkaloid
B. pinnatum	36.27 ± 0.40^{d}	45.69 ± 0.12^{b}	62.81 ± 0.22^{b}	37.58 ± 0.11^{d}	31.99 ± 0.25^{b}	35.05 ± 0.25^{c}	69.94 ± 0.14^{a}	29.21 ± 0.18^{b}
A. cordifolia	43.24 ± 0.40^{a}	48.31 ± 0.10^{a}	64.10 ± 1.01^{a}	44.96 ± 0.09^{b}	22.17 ± 0.13^{c}	37.59 ± 0.25^{a}	60.88 ± 0.07^{b}	28.19 ± 0.23^{c}
A.wilkesiana	38.50 ± 0.26^{c}	48.60 ± 0.08^{a}	66.81 ± 0.10^{a}	43.21 ± 0.16^{c}	32.77 ± 0.29^{b}	36.24 ± 0.21^{b}	43.59 ± 0.09^{c}	30.40 ± 0.13^{a}
C. lanatus	40.35 ± 0.53^{b}	43.91 ± 0.13^{c}	60.37 ± 0.18^{c}	78.36 ± 0.16^{a}	78.99 ± 0.21^{a}	32.76 ± 0.33^{d}	43.20 ± 0.05^{d}	-

(Mean values with different superscripts were significant; $p<0.05$)

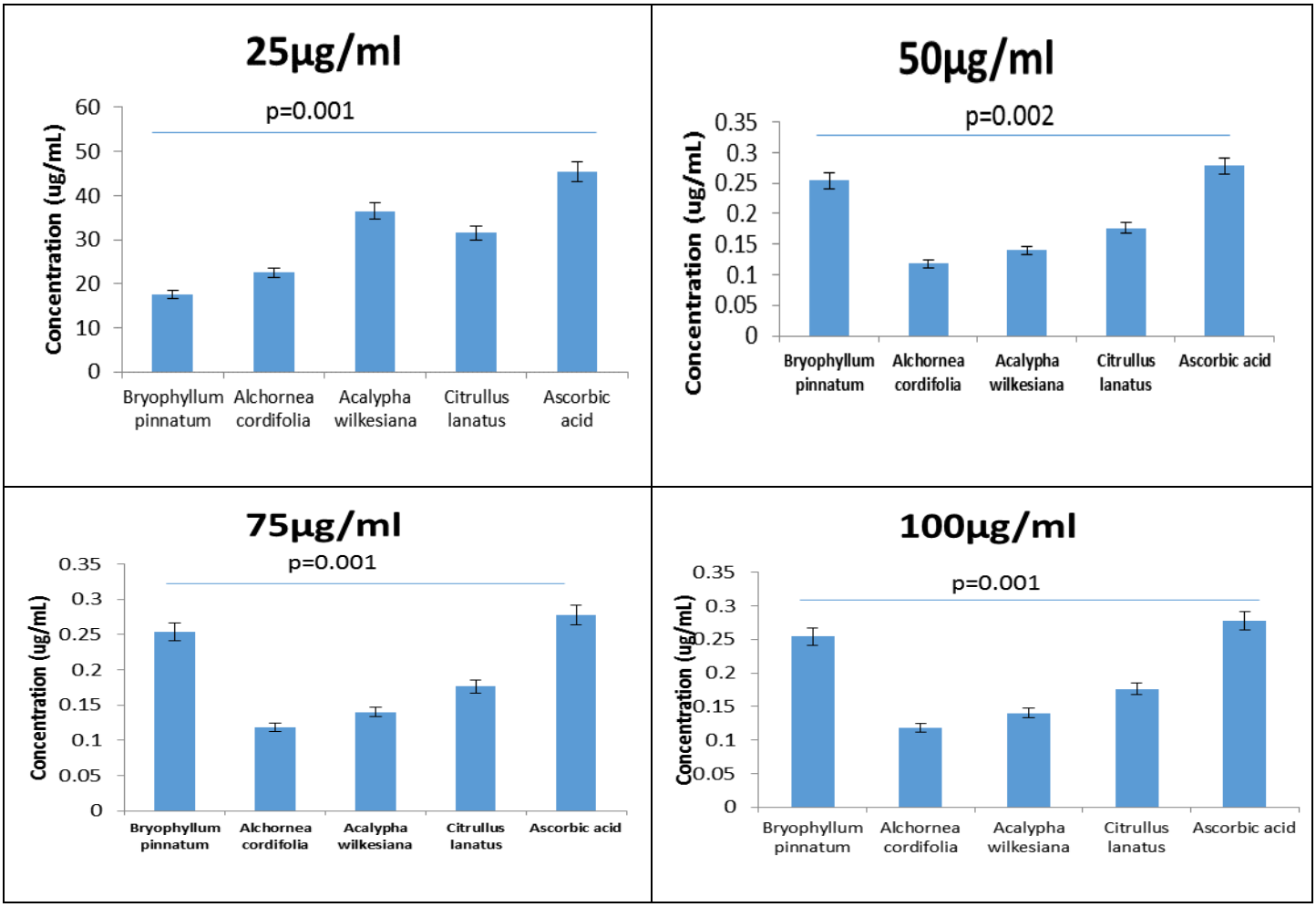

Fig 1; 2,2-diphenyl-1-picrylhydrazyl radical scavenging of the plant extracts (error bar indicates differences in standard error mean)

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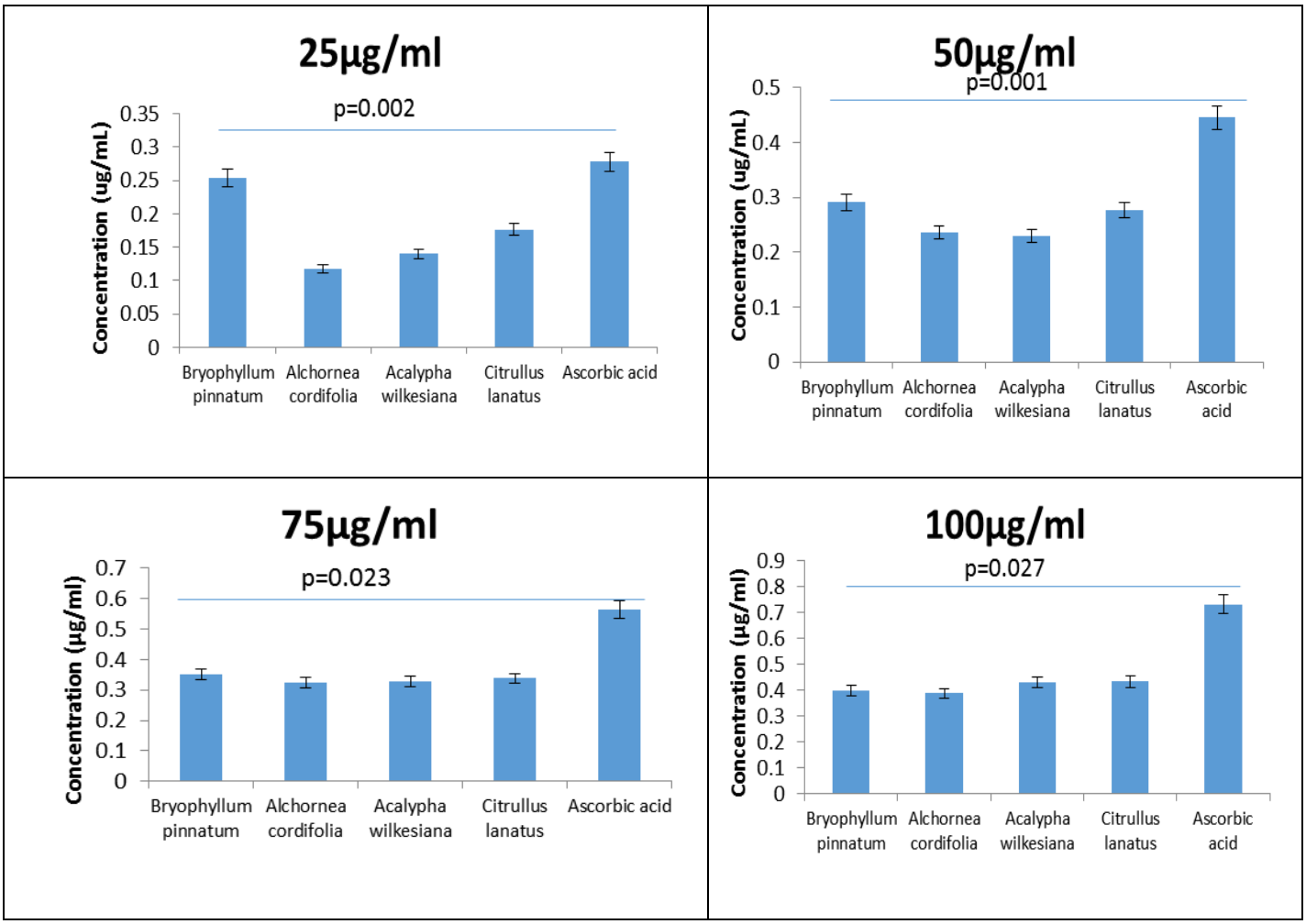

Fig 2; Ferric reducing antioxidant power activity of the extracts (error bar indicates differences in standard error mean)

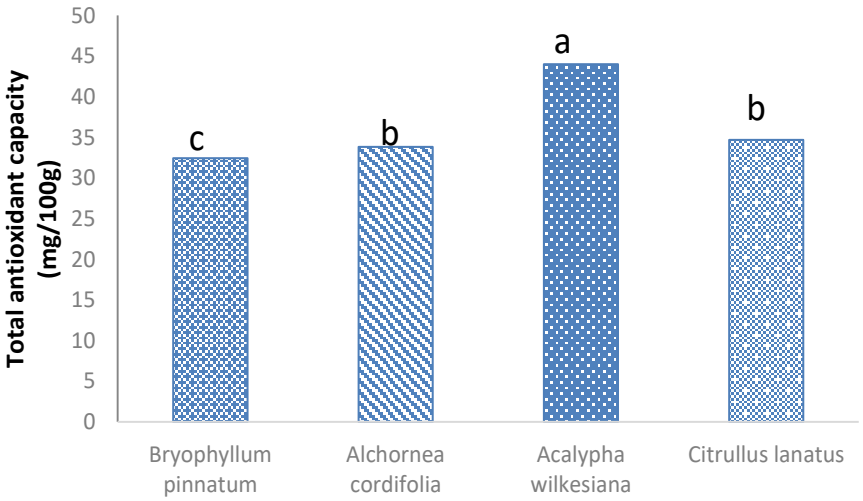

Fig 3; Total antioxidant capacity of the extracts (Mean values with different superscripts were significant; $p<0.05$)

(0.352±0.002) relative to the other leaf extracts. At 100 µg/ml concentration, *A. wilkesiana* (71.50±0.50) and *C. lanatus* (0.434±0.004) exhibited significantly (P<0.05) more FRAP activity relative to those of *A. cordifolia* and *B. pinnatum*. Comparatively more (P<0.05) total antioxidant activity (TAC) was observed in *A. wilkesiana* while *A. cordifolia* and *C. lanatus* were similar (P>0.05) in TAC while *B. pinnatum* had the lowest TAC (Fig 3).

Antimicrobial activities: The inhibitory activities (zone of inhibition) of the four plant extracts ranged from 15.25±0.14 to 40.50±0.65mm for *Streptococcus faecalis* and 15.63±0.24 to 16.13±0.31mm for *Pseudomonas aeruginosa*. *C. lanatus* extracts did not inhibit *Streptococcus faecalis* and *Pseudomonas aeruginosa* at any of concentration while the organisms did not show any sensitivity to distilled water used as the negative control (Table 3). *Streptococcus faecalis* showed significant susceptibility to *A. wilkesiana and A. cordifolia* leaf extracts at 100mg/ml concentration in a manner similar to inhibition observed with Levofloxacin antibiotic. The inhibition zones of *A. wilkesiana, A. cordifolia* and Levofloxacin agai*nst S. faecalis* increased with increase in concentration, reaching 40.50±0.65, 40.50±0.20 and 40.38±0.24 mm at 400 mg/ml respectively. Generally, *Pseudomonas aeruginosa* was less sensitive to the plant extracts compared to Levofloxacin. The susceptibility of *P. aeruginosa* to *A. wilkesiana* and *A. cordifolia* leaf extracts was observed only at 400 mg/ml concentration with inhibition zones of 15.63±0.24 and 16.13±0.31 mm respectively. Among the plant extracts, *A. cordifolia* leaf extract at MIC 0.16mg/ml showed highest inhibition to *S. faecalis* whereas the least inhibition was observed in *A. wilkesiana* leaf extract against *P. aeruginosa* at MIC of 2.56 mg/ml.

4. DISCUSSION

Increasing rate of antimicrobial resistance in aquaculture ranging from bacteria resistance to commonly used antibiotics [38] to high level antibiotic residues in feeds and fish products [39] are major concern to fish production and human consumption. Use of alternative source of antimicrobial agents particularly from plant extracts has improved therapeutic management, production and healthy consumption of fish products [40]. From the study, the preliminary phytochemical profile of the ethanolic extract of *Bryophyllum pinnatum, Alchornea cordifolia* and *Acalypha wilkesiana* leaves, and *Citrullus lanatus* seeds revealed high level bioactive components in all the extract providing a large scale opportunity for harnessing these secondary metabolites for medical purposes. The detection of terpenoids and tannins is evident in all the extract. These metabolites possess analgesic and anti-inflammatory activities that could be utilized for therapeutic management [41]. Tannins have been reported to speed up the recovery from injuries and inflamed mucous membranes [21]. All four medicinal plant extracts included large amounts of steroids, which often enhance the action of several sex hormones [42]. Saponins are also known to damage the lipopolysaccharide structural components of Gram positive bacteria's outer phospholipidic membrane, allowing lipophilic solutes to pass through and render the cell wall inert [21]. Thus, they are good candidates for antimicrobial agents with effective inhibitory properties against microbial invasion [18,43]. Protection from allergies, inflammation, free radicals, platelet aggregation, bacteria, ulcers, hepatoxins, viruses, and cancers are just a few of the biological effects of flavonoids [18]. Presence of alkaloids in three out of four selected medicinal plant extracts is a promising antimicrobial source with ability to inhibit the bacteria extra-outer membrane and DNA synthesis [18]. One of the probable resistance mechanisms is the ability of components of cardiac glycosides, which are condensed products of sugars with various organic hydroxyl compounds or its derivatives, to efficiently inhibit the efflux pump function of various bacteria [14,44].

High antioxidant properties of these extracts have been identified as free radical or active oxygen scavengers [45] with known anti-inflammatory actions [10,46]. It has been suggested that the antimicrobial actions of these compounds are related to their anti-oxidant property [47] and their effects on the health of human and animal are highly beneficial as previously described [18]. Additionally, it has been noted that these phytochemical compounds act as anti-oxidative agents, scavenging for available molecular oxygen (O_2) needed for respiration or oxidizing nutrients to produce energy for bacterial pathogens and producing reactive oxygen by-products like superoxide anion radicals (O_2-) and highly reactive hydroxyl radicals (•OH), which are continuously produced during aerobic processes. These compounds result from numerous membrane-associated respiratory chain enzymes sequentially catalyzing univalent reductions of molecular oxygen. [48,49]. The biological effect of anti-oxidation of phytochemicals is more adverse on DNA, RNA, proteins and lipids metabolic pathways resulting in inhibition of cell wall synthesis, organelles maturation and protein synthesis for bacteria cell replication [50].

The inhibitory activities of the ethanolic extracts of *Bryophyllum pinnatum, Acalypha wilkesiana* and *Alchornea cordifolia* leaves against *Streptococcus faecalis* further affirm the potential antimicrobial properties of these extracts to be utilized as therapeutic option in bacterial infection and disease management. Activity of the antioxidant of the tested plant extract in the present study revealed another avenue for the development of effective antioxidant that could aid the clean-up of oxygen free radicals which could lead to inflammation and physiological toxicity.

5. CONCLUSION

The study affirms the antioxidant and antimicrobial potentials of ethanolic extracts of *Bryophyllum pinnatum, Alchornea cordifolia* and *Acalypha wilkesiana* leaves, further substantiate nutraceutical potentials of these plants. *Alchornea cordifolia* and *Acalypha wilkesiana* are important sources of phenolic compounds that can be harnessed as lead compounds for development of antibacterial agents and natural source of antioxidants for commercial food products and drugs. However, further in vitro kinetic studies and in vivo antioxidative and antibiotic activities of these extracts in various commercial fish samples are needed to validate their applications in aquaculture.

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Table 3; Inhibitory activities of the extracts

Plant extracts	Assay Organisms	Inhibitory concentration				**MIC (mg/ml)**	**P value**
		400 mg/ml	200 mg/ml	100 mg/ml	Distilled water		
Bryophyllum pinnatum	SF	21.38±0.24	15.25±0.14	0.00	0.00	0.64	0.023
	PA	0.00	0.00	0.00	0.00	0.00	
Acalypha wilkesiana	SF	40.50±0.65	30.75±0.48	25.25±0.25	0.00	0.64	0.36
	PA	15.63±0.24	0.00	0.00	0.00	2.56	
Citrullus lanatus	SF	0.00	0.00	0.00	0.00	0.00	
	PA	0.00	0.00	0.00	0.00	0.00	
Alchornea cordifolia	SF	40.50±0.2	35.50±0.29	31.50±0.29	0.00	0.16	0.041
	PA	16.13±0.31	0.00	0.00	0.00	0.64	
		Inhibitory concentration					
		50 µg/ml	25 µg/ml	12.5 µg/ml			
Levofloxacin	SF	40.38±0.24	36.50±0.29	31.38±0.24	0.00	0.005	0.01
	PA	29.13±0.31	23.38±0.24	19.63±0.24	0.00	0.005	0.02

(SF, *Streptococcus faecalis;* PA, *Pseudomonas aeruginosa*, MIC, Minimum inhibitory concentration; $p<0.05$, significant)

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