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Antimicrobial, antioxidant and cytotoxic evaluation of two underutilised food plants: *Averrhoa bilimbi* L. (Oxalidaceae) and *Phyllanthus acidus* L. Skeels (Phyllanthaceae)

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ABSTRACT

Averrhoa bilimbi (P1) and *Phyllantus acidus* (P2) are traditionally used in the management of various diseases in several cultures. Leaf (L) and fruit (F) extracts (decoction, hexane, dichloromethane, ethyl acetate- EtOAc and methanol) were prepared and investigated for their antimicrobial, antioxidant and cytotoxic properties *in vitro*. P1 and P2 extracts showed broad spectrum activity against ATCC bacterial strains and clinical isolates. P1L and P2F EtOAc extracts inhibited the growth of ATCC strains of *S. aureus* and *V. parahaemolytius* (MIC = 250 µg/ml). P1F EtOAc extract was found active against ATCC strains of *E. coli* and *S. aureus* (MIC = 250 µg/ml). P1F was active against all tested clinical isolate strains with maximum inhibition against *S. aureus* ((MIC = 130 µg/ml). Hexane and EtOAc extracts of P2F showed activity against clinical isolate strains of *S. aureus* ((MIC = 130 and 250 µg/ml, respectively). P2F hexane extract displayed antimicrobial activity against MRSA at MIC value similar to that of streptomycin (250 µg/ml). Furthermore, the antioxidant activity of the different extracts was assessed using DPPH, ABTS and the FRAP assay. The cytotoxicity of the decoction and methanol extracts were also determined using normal adult African green monkey kidney cells (Vero cells). Leaf decoction of P1 showed DPPH radical scavenging activity at an IC_{50} of 5.30 µg/ml, which was comparable to the positive control, ascorbic acid (IC_{50}: 5.89 µg/ml). Leaf extracts of P1 were better hydrogen donors compared to leaf and fruit extracts of P2. The methanol and decoction extracts of P1L showed low activity against the ABTS cation at an IC_{50} value of 39.26 and 31.68 µg/ml, respectively. The P2L and P2F extracts were unable to scavenge ABTS cation radicals. The FRAP values for P2L and P2F extracts were in the range of 59.83–120.89 and 23.98–90.71 TE µM, respectively. Decoction and methanol extracts showed no toxicity at the highest concentration tested. In conclusion, data amassed from the present investigation could open new avenues for research and may serve as the basis for the development of novel drugs.

1. Introduction

Human beings have always made use of their native flora, as a source of nutrition as well as for fuel, medicine, clothing, dwellings, and chemical production. Traditional knowledge of plants and their properties has always been transmitted from generation to generation through the natural course of everyday life (Polat et al., 2013). Research on the traditional use of medicinal plants has attained considerable interest within the scientific community in recent years. Use of folk medicine has increased considerably in industrial countries, as numerous drugs have been derived from tropical flora (Bibi et al., 2014).

Averrhoa bilimbi is an edible and underutilized fruit from the Oxalidaceae family (Mokhtar and Aziz, 2016). The leaf of the plant is traditionally used as paste against itches, swelling, skin eruptions and coughing. Fruit decoctions are used to treat inflammatory conditions such as hepatitis, fever and diarrhea. In India, the fruit is used against obesity (Mokhtar and Aziz, 2016). The leaves of the plant are used in the treatment of intestinal infections, fever, skin infections and hypertension among the people of the Mascarene Island (Gurib-Fakim and

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Gueho, 1996). Fruit extracts of *A. bilimbi* were found to show antifungal activity against *A. niger* and *C. albicans* (Rana et al., 2014). The fruit extract of *A. bilimbi* showed hypotriglyceridemic, anti-lipid peroxidative, and anti-atherogenic activities in streptozotocin induced diabetic rats. The methanolic fruit extract showed cytotoxic activity against MCF-7 human breast cancer cell lines (Nair et al., 2016).

Phyllanthus acidus belongs to the Phyllanthaceae family. The plant has been traditionally used to treat inflammatory and oxidative stress related ailments including rheumatism, bronchitis, asthma, diabetes, respiratory problems, gonorrhea and hepatic disease (Chakraborty et al., 2012). This species is also utilized as sudorific, to improve eyesight, memory and in the treatment of cough, psoriasis, as well as skin disorders. The leaves of the plant have been reported to be effective against hypertension. The leaf, bark and roots are used for the treatment of fevers (Chakraborty et al., 2012). The people of the Mascarene Island use the plant against hepatitis and dysentery (Gurib-Fakim and Gueho, 1996). Methanol extracts of the leaves and fruits have been reported to show antimicrobial and hepatoprotective activity (Chakraborty et al., 2012). Methanol fruit extracts have also been reported to exhibit cytotoxic and antioxidant potential (Moniruzzaman et al., 2015).

Studies have demonstrated that *A. bilimbi* and *P. acidus* show various biological activities including antioxidant, antimicrobial, antidiabetic, antihyperlipidemic (Kumar et al., 2013 and Andrianto et al., 2017). However, to date no attempt has been made to study these underutilised plants grown under the current climatic conditions prevailing in Mauritius. Indeed, it has been argued that the biological activities of plants vary on external factors such as geographical location and climate amongst others. Interestingly, several plants growing on the tropical island of Mauritius have previously been found to be unique in terms of their pharmacological properties. Therefore, the present study was designed to evaluate the antimicrobial, antioxidant and cytotoxic activities of the fruit and leaf extracts of *A. bilimbi* and *P. acidus*. It is anticipated that data amassed herein will help to validate their traditional claims as well as delineate further health benefits so as to encourage their use as functional foods.

2. Materials and methods

2.1. Extraction

Fresh fruits and leaves of *Averrhoa bilimbi* and *Phyllanthus acidus* were collected from the Northern region of Mauritius. The plant was identified by the local botanist. The leaves were dried at 40 °C in a drying cabinet for 4–5 days until constant mass was obtained. Dried powdered sample was macerated using hexane, dichloromethane (DCM), ethyl acetate and methanol (10:1 solvent to dry weight ratio) respectively (Rangasamy et al., 2007). For the decoction, 50 g of dried plant powder was extracted with 200 ml of distilled water at 100 °C until the volume decreased to a quarter of the original volume. The extracts were filtered, combined and dried under reduced pressure using a rotatory evaporator.

2.2. Antimicrobial activity

2.2.1. Microbial strains

The antimicrobial activity of crude extracts were evaluated using American Type Culture Collection (ATCC) namely *Escherichia coli* (ATCC 25922) *Pseudomonas aeruginosa* (ATCC 27853), *Propionibacterium acnes* (ATCC 11827 and 6919), *Staphylococcus aureus* (ATCC 25923), *Salmonella typhimurium* (ATCC 14028), *Vibrio parahaemolytius* (ATCC 17802) and clinical laboratory isolates such as *Acinetobacter spp.*, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella spp.*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Proteus spp.*, Methicllin-Resistant *Staphyloccocus aureus* (MRSA), and *Staphylococcus aureus*. The clinical isolates were obtained from Victoria Hospital, Candos, Mauritius.

2.2.2. Micro-dilution broth susceptibility assay

A two-fold serial microdilution technique was used to determine the minimum inhibitory concentration values for the different extracts as described by Shai et al. (2008) with some modification. One hundred microliters of each extract (16 mg/ml) was serially diluted two-fold in triplicate with sterile peptone water in a 96-well microtitre plate. Fresh bacterial and fungal inoculums were prepared and adjusted to 0.5 McFarland which were further diluted 1:100 with fresh sterile peptone water broth to yield starting inoculums of approximately 1 x 10^6 CFU/ml. One hundred microliters of bacterial culture was added to each well of bacterial plates. Chloramphenicol, ciprofloxacin, tetracycline and streptomycin were used as positive controls for bacteria. A negative control included sterile peptone water broth. The bacterial plates were incubated at 37 °C for 24 h. After incubation, 40 µl of iodo-nitrotetrazolium chloride (0.2 mg/ml) was added to each well and the plates were further incubated for 20 min. Bacterial growth was denoted by red coloration. The well of lowest concentration in which no pinkish red coloration was observed was considered to be the minimum inhibitory concentration (MIC). The total activity (TA ml/g) value was calculated as the total mass extracted from 1 g of plant material divided by the MIC value (mg/ml).

2.3. Antioxidant activity

2.3.1. Radical scavenging activity using DPPH$^{\cdot}$ method

The free radical scavenging capacity of extracts was spectrophotometrically assessed using DPPH. Samples (400 µg/ml, 100 µl) were serially diluted using methanol (100 µl) in a 96-well microtiter plate. Methanolic solution of DPPH (100 µM, 200 µl) was added to each well and the plate was incubated at 37 °C for 30 min. The experiments were performed in triplicate and the absorbance was measured at 517 nm. Ascorbic acid and Trolox were used as positive control and methanol as the blank. The percentage inhibition was calculated as follows; percentage inhibition = $[(A_0 - A_1)/A_0]$ x 100, where A_0 is the absorbance of the blank sample and A_1 is the absorbance of the sample.

2.3.2. ABTS cation radical scavenging activity

The total antioxidant activity of the extracts was determined with slight modification according to Saeed et al. (2012). The ABTS cation radical was generated by reacting potassium persulfate solution (15 ml, 2.45 mM) with ABTS solution (15 ml, 7 mM) and kept overnight in the dark to yield a dark blue solution. The concentration of the resulting blue $ABTS^{\cdot+}$ was adjusted to an absorbance of 0.70 ± 0.02 at 734 nm by diluting with ethanol. Sample (400 µg/ml, 10 µl) was added to the resulting blue green ABTS cation radical solution (190 µl) in a 96 well microplate. The decrease in absorbance was measured at 734 nm for 6 min. Ascorbic acid and Trolox were used as positive control and ethanol as blank. The percentage inhibition was calculated according to the formula: percentage inhibition = $[(A_0 - A_1)/A_0]$ x 100, where A_0 is the absorbance of the blank sample and A_1 is the absorbance of the sample.

2.3.3. Ferric reducing antioxidant power (FRAP) assay

The reducing capacity of the extracts was determined through FRAP assay according to a modified method of Benzie and Strain (1996). FRAP reagent was freshly prepared by mixing 10 mM of TPTZ in 40 mM hydrochloric acid, 20 mM $FeCl_3.6H_2O$ in distilled water and 300 mM acetate buffer pH 3.6. The different extracts (30 µl) were added to a 96 well microtitre plate followed by FRAP reagent (270 µl). Ascorbic acid was used as positive control. A calibration curve was prepared by plotting the absorbance at 593 nm versus different concentrations of $FeSO_4$. The concentrations of $FeSO_4$ were in turn plotted against concentration of standard antioxidant Trolox. The FRAP values were obtained by comparing the absorbance change in the test mixture with

those obtained from increasing concentrations of Fe^{3+} and the results were expressed in mM trolox equivalent (TE) for 100 mg/L extract.

2.4. Cell culture

The Vero cell line was maintained in culture flasks containing Eagle's Minimum Essential Medium supplemented with 1% antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin and 250 µg/mL fungizone) and 10% heat-inactivated fetal bovine serum (FBS). The cells were grown in a humidified incubator set at 5% CO_2 and 37 ºC. After the formation of a monolayer, cells were sub-cultured. The cells were detached by treating them with trypsin-EDTA (0.25% trypsin containing 0.01% EDTA) for 10 min followed by the addition of supplemented media to inhibit the reaction.

2.4.1. In vitro cytotoxicity assay

The cytotoxicity was determined using the XTT (2, 3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) method according to the XTT Cell Proliferation Kit II and method described by Zheng et al., (2001). Briefly, 100 µL of Vero cells were seeded in a sterile 96-well microtitre plate (concentration 1×10^5 cells/mL) and incubated at 37 °C and 5% CO_2 for 24 h to allow cellular attachment to the bottom of the wells. Test extracts were prepared to a stock solution of 20 mg/mL in 100% DMSO, added to the microtitre plate and serially diluted to yield a concentration range from 400-1.563 µg/mL. The final concentration of DMSO was 2% in the test plate. The microtitre plate was incubated for a further 72 h. The control wells included vehicle-treated cells exposed to 2% DMSO and the positive control, Actinomycin D, with concentrations ranging between 0.5 and 0.002 µg/mL. After the 72 h incubation period, 50 µL of the XTT reagent was added to a final concentration of 0.3 mg/mL and the plate was then further incubated for another 2 h. The absorbance of the colour complex was read at 490 nm with a reference wavelength set at 690 nm using a BIOTEKPower-Wave XS multi-well plate reader. The assay was performed in triplicate to calculate an IC_{50} of the cell population for each of the samples.

2.5. Statistical analysis

All determinations were carried out in triplicates and the results were reported as mean ± standard deviation. Calculation of IC_{50} was done using GraphPad Prism Version 5.03 for Windows (GraphPad Software Inc.).

3. Results and discussion

3.1. Antimicrobial activity

In this study, the antimicrobial activities of extracts of two different plant species prepared in different solvents were evaluated against ATCC as well as clinical isolates. The results are represented as MIC (mg/ml) and total activity (ml/g) (Tables 1 and 2). Most extracts showed a broad antibacterial spectrum against the tested strains. The MIC values of the extracts ranged from 0.50 to 4.00 mg/ml for decoction extracts while the activity was higher with organic extracts (MIC range: 0.13–4 mg/ml) against both Gram positive and Gram negative bacteria. All extracts from *Averrhoa bilimbi* (P1) fruit were active against ATCC strains of *S. aureus*, *S. typhimurium, E. coli* and *P. aeruginosa* and *V. parahaemolytius*. All leaf extracts of *Phyllanthus acidus* (P2) revealed inhibitory activity against *E. coli* and *P. aeruginosa*. Hexane extracts showed low activity against most tested strains (MIC range: 2.00-4.00 mg/ml). All P2 fruit extracts showed activity against all tested clinical isolate strains (MIC range: 0.13-4.00 mg/ml). Ethyl acetate fruit extract of *Phyllanthus acidus* (P2F EtOAc) showed notable activity against *Acinetobacter* spp. with MIC values of 0.25 mg/ml respectively while P2F dichloromethane (P2F DCM) and P2F ethyl acetate (P2F EtOAc) extracts revealed considerable activity at MIC values of 0.50 mg/ml. P2F hexane extracts showed significant activity against *S. aureus* (MIC: 0.13 mg/ml). P2F EtOAc revealed antibacterial potential similar to ciprofloxacin against *Klebsiella* spp. with MIC value of 0.25 mg/ml. Lowest MIC value of 0.25 mg/ml was observed with P1L EtOAc extracts against *E. faecalis* strain. P1L EtOAc and P1F EtOAc inhibited the growth of *Proteus* spp. at MIC value of 0.25 mg/ml. P1F EtOAc was also active against *P. mirabilis* (MIC: 0.25 mg/ml) and the activity was higher than streptomycin (MIC: 0.50 mg/ml). Among the 20 extracts tested, 6 extracts showed noteworthy activity against MRSA (MIC: 0.25–0.50 mg/ml) while P2F hexane extract showed similar activity as streptomycin (MIC: 0.25 mg/ml). *A. bilimbi* methanolic and chloroform fruit extracts have been reported to inhibit *S. aureus*, *B. subtilis*, *K. pneumonia* and *S. marcescens* at a concentration of 50, 100 and 150 mg *via* disk diffusion method (Abraham, 2016). This species has been used to treat fever, healing blisters, pimples, anal disorders, boils, arthritis, intestinal disorders, cough and cold, hypertension and as soft drink (Abraham, 2016). Leaf and fruit extracts showed a wide range of phytochemicals such as saponins, coumarins, flavonoids and quinones (Abraham, 2016). *Phyllanthus acidus* (P2) extracts showed broad spectrum antibacterial activity against ATCC as well as clinical isolate strains. The antibacterial potential of the leaf and fruit extracts could be due to the presence of various phytochemicals such as saponins, coumarins, flavonoids, quinone and anthocyanins. Tannins and polyphenols were present only in the leaf extracts. Methanolic fruit extract showed significantly antibacterial activity against *E. coli*, *Salmonella* spp. *B. cereus* and *S. aureus* at 100 µg using disc diffusion method (Rana et al., 2014). Reported compounds from *P. acidus* include 4-hydroxybenzoic acid, caffeic acid, adenosine, kaempferol and hypogallic acid (Jagessar and Hope, 2016). 4-hydroxybenzoic acid has been reported to show antimicrobial activity against Gram-positive and Gram-negative bacteria such as *E. coli*, *Bacillus aureus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Lactobacilli* and *Listeria monocytogenes* (Manuja et al., 2013). Caffeic acid displayed antibacterial properties but has some issues regarding solubility and stability to environment stress (Pinho et al., 2015). Pinho et al. (2015) showed that the antibacterial activity of caffeic acid was significantly increased by cyclodextrins encapsulation. This plant has been traditionally used to treat inflammatory and oxidative stress related ailments including rheumatism, bronchitis, asthma, diabetes, respiratory problems, gonorrhea and hepatic disease. This species is also utilized to improve eyesight, memory and in the treatment of cough, psoriasis, skin disorders and used as sudorific. The leaf, bark and roots are used against fever (Chakraborty et al., 2012). *Phyllanthus* genus contain a wide range of secondary metabolites such as alkaloids, tannins, flavonoids, lignans, phenolics and terpenes which could be related to the antimicrobial activity of the plant. These compounds may serve as defense mechanism against various microorganisms (Jagajothi et al., 2013).

3.2. Antioxidant activity

Oxidative stress is an important risk factor in the pathogenesis of numerous chronic diseases (Badakhshan et al., 2012). Free radicals and other reactive oxygen species are recognized as agents involved in the pathogenesis of sicknesses such as asthma, inflammatory arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis. Reactive oxygen species are also said to be responsible for the human aging (Badakhshan et al., 2012). Extracts were tested for their antioxidant potential using DPPH assay. Hexane and dichloromethane extracts as well as P1F EtOAc, P1F decoction, P2L ETOAc, P2F EtOAc and P2F decoction were unable to scavenge $DPPH^{\bullet}$ radical (Percentage inhibition < 30%). Yan et al. (2012) revealed that the fruit extract of P1 showed the presence of Vitamin A and correlation tests indicated that samples with high vitamin A did not possess high antioxidant activity. A review on *A. bilimbi* species showed that the fruit extracts contain flavonoids, saponins and triterpenoid and the chemical

Table 1
Antimicrobial activity of crude extracts against ATCC strains.

MIC (mg/ml)								
Plant	Extract	EC	PA	Pa^1	Pa^2	SA	ST	VB
P1 L	Dec	-	4.00 (24.60)	-	-	2.00 (49.20)	-	-
	Hex	4.00 (2.67)	4.00 (2.67)	NT	NT	4.00 (2.67)	4.00 (2.67)	-
	DCM	4.00 (1.66)	4.00 (1.66)	NT	NT	4.00 (1.66)	2.00 (3.32)	4.00 (1.66)
	EtOAc	2.00 (9.05)	0.50 (36.20)	NT	NT	0.25 (72.40)	1.00 (18.1)	0.25 (72.4)
	MeOH	2.00 (59.67)	2.00 (59.67)	-	-	0.50 (238.6)	1.00 (119.3)	0.50 (238.6)
P1 F	Dec	4.00 (12.78)	4.00 (12.78)	-	-	2.00 (25.55)	4.00 (12.78)	2.00 (25.55)
	Hex	4.00 (5.08)	4.00	NT	NT	4.00 (5.08)	1.00 (20.30)	-
	DCM	-	-	NT	NT	4.00 (4.53)	4.00 (4.53)	4.00 (4.53)
	EtOAc	0.25 (172.4)	0.50 (86.20)	NT	NT	0.25 (172.4)	4.00 (10.78)	4.00 (10.78)
	MeOH	2.00 (155.42)	2.00 (155.42)	-	-	1.00 (310.83)	2.00 (155.42)	2.00 (155.42)
P2 L	Dec	4.00 (11.15)	2.00 (22.30)	-	-	4.00 (11.15)	4.00 (11.15)	-
	Hex	4.00 (3.90)	4.00 (3.90)	NT	NT	-	2.00 (7.81)	4.00 (3.90)
	DCM	4.00 (2.61)	2.00 (5.21)	NT	NT	2.00 (5.21)	0.50 (20.84)	1.00 (10.42)
	EtOAc	1.00 (5.05)	1.00 (5.05)	NT	NT	1.00 (5.05)	1.00 (5.05)	1.00 (5.05)
	MeOH	2.00 (33.99)	2.00 (33.99)	-	-	1.00 (252.9)	1.00 (67.97)	1.00 (67.97)
P2 F	Dec	2.00 (29.60)	2.00 (29.60)	-	-	1.00 (59.20)	4.00 (14.80)	2.00 (29.60)
	Hex	2.00 (3.73)	2.00 (3.73)	NT	NT	0.50 (14.92)	4.00 (1.87)	1.00 (7.46)
	DCM	1.00 (42.30)	1.00 (42.30)	NT	NT	0.50 (84.60)	1.00 (42.30)	0.50 (84.60)
	EtOAc	1.00 (83.36)	0.50 (166.5)	NT	NT	0.25 (333.4)	1.00 (83.36)	0.25 (333.4)
	MeOH	2.00 (252.97)	2.00 (252.9)	-	-	1.00 (505.93)	4.00 (126.48)	2.00 (252.97)
CIP*		0.0039	0.025	NT	NT	0.0002	0.0008	0.0002
CHL*		0.0004	0.016	NT	NT	0.0078	0.0031	0.0008
STR*		0.0156	0.250	NT	NT	0.0039	+	+
TET*				0.0015	0.0008			

P1: *Averrhoa bilimbi*; P2: *Phyllanthus acidus*; L: leaf; F: fruit; MIC: Minimum inhibitory concentration; DCM: dichloromethane; EtOAc: ethyl acetate; MeOH: methanol; ATCC strains: EC*: Escherichia coli* ATCC 25922; PA: *Pseudomonas aeruginosa* ATCC 27853; Pa^1: *Propionibacterium acnes* ATCC 11827; Pa^2: *Propionibacterium acnes* ATCC 6919; ST: *Salmonella typhimurium* ATCC 14028; SA: *Staphylococcus aureus* ATCC 25923; VP: *Vibrio parahaemolytius* ATCC 17802. *Positive control for bacteria; CHL: Chloramphenicol; CIP: Ciprofloxacin; STR: Streptomycin; TET: tetracycline; ( ): total activity in ml/g; -: no activity; +: no bacterial growth at a stock concentration of 10 mg/ml; NT: not tested. All data represents the mean of three independent experiments.

constituents include amino acids, citric acid, cyaniding-3-O-h-D-glucoside, phenolics, potassium ions, sugars and vitamin A (Kumar et al., 2013). A review conducted by Alhassan and Ahmed (2016) showed that the fruits extracts of P1 exhibited strong DPPH radical scavenging activity with an IC_{50} value of 20.35 µg/ml. The chemical constituents of plants vary depending on the species, variety and part of the plant, with conditions of growth (soil, water and temperature), and with the age of the plant. The phytochemical compositions of the plant also vary according to the geographical regions, season and time of collection and different climatic conditions which could explain the difference in antioxidant activity of similar species from other regions (Arya et al., 2010). P1 leaf decoction showed significant DPPH radical scavenging activity at IC_{50} 5.30 µg/ml which was comparable to the positive control, ascorbic acid (IC_{50}: 5.89 µg/ml). Extracts were also tested against two different methods namely ABTS, and FRAP assays. The reducing capacity of a compound may serve as a significant indicator of its potential anti-oxidant activity (Mandal et al., 2011). FRAP assay measures the reducing potential of an anti-oxidant reacting with a ferric tripyridyltriazine [Fe3+-TPTZ] complex and produce a colored ferrous tripyridyltriazine [Fe2+-TPTZ]. Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom (Nishaa et al., 2012). All extracts from P1F, P2L and P2F were found inactive against ABTS cation radical. Methanol and decoction extracts from P1L showed moderate activity against ABTS cation radical (IC_{50} range: 31.68-39.26 µg/ml). P1L extracts showed higher FRAP value compared to P1F extracts. Phytochemical analysis of leaf extracts of P1 showed the presence of sugars, protein, aldehyde, cardiac glycosides, flavonoids, phenols, alkaloids, tannins and coumarins. Phytochemicals such as flavonoids, tannins and coumarins are potent antioxidants (Valsan and Raphael, 2016) which could explain the reducing potential of P1 leaf extracts (Table 3).

3.3. Cytotoxicity of decoction and methanol extracts

Decoction and methanol extracts showed higher antimicrobial and antioxidant activities and thus their cytotoxicity were determined using normal adult African green monkey kidney cells (Vero cells). P1 and P2 extracts demonstrated no toxicity at the highest concentration tested, 400 µg/ml. Medicinal plants synthesize toxic substances which in nature protect them against insects, infections and herbivores but can also affect the organisms that feed on them (Varalakshmi et al., 2011). Thus, cytotoxicity testing is important to assess and validate the safety of medicinal plants for traditional use (Amer, 2014). *Phyllanthus acidus* leaf and fruit decoction as well as methanol extracts showed low cytotoxic effects against vero cells. The leaf decoction is used by tribal healers in Bangladesh for the treatment of liver disease (Jain and Singhai, 2011). The hepatoprotective effects of crude ethanolic and aqueous leaf extracts of *P. acidus* were investigated on acetaminophen (APAP) and thioacetamide (TAA) induced liver toxicity in Wistar rats. The extracts prevented the increase of serum AST, ALT, ALP and total bilirubin. The elevated levels of AST and ALT showed hepatocytes damage or alterations in the membrane permeability showing the severity of hepatocellular damage induced by APAP and TAA which was indicated by APAP and TAA treated control groups. The aqueous extract was more potent compared to the ethanolic extract and thus showed significant hepatoprotective behaviour on APAP and TAA induced hepatotoxicity (Jain and Singhai, 2011). *Averrhoa bilimbi* decoction and methanol extracts showed low cytotoxicity. Infusion of the leaves of the plant is traditional used against intestinal infections while leaf cataplasm is effective in the treatment of skin diseases. The fruit juice as well as the leaf decoction is consumed against hypoglycemia (Gurib-

Table 2
Antimicrobial activity of crude extracts against clinical isolates.

MIC (mg/ml)										
Plant	Extract	EC	PA	SA	AC	KL	EF	PR	PM	MRSA
P1 L	Dec	1.00 (98.40)	-	2.00 (49.20)	-	-	-	-	-	4.00 (24.60)
	Hex	4.00 (2.67)	4.00 (2.67)	4.00 (2.67)	2.00 (5.34)	4.00 (2.67)	4.00 (2.67)	4.00 (2.67)	4.00 (2.67)	-
	DCM	4.00 (1.66)	4.00 (1.66)	4.00 (1.66)	2.00 (3.32)	4.00 (1.66)	4.00 (1.66)	4.00 (1.66)	4.00 (1.66)	-
	EtOAc	2.00 (9.05)	-	0.50 (72.40)	-	2.00 (9.05)	0.25 (72.40)	0.25 (72.40)	4.00 (4.53)	0.50 (36.20)
	MeOH	1.00 (119.3)	1.00 (119.3)	0.25 (477.32)	4.00 (29.83)	2.00 (59.67)	2.00 (59.67)	4.00 (29.83)	4.00 (29.83)	0.50 (238.6)
P1 F	Dec	4.00 (12.78)	4.00 (12.78)	1.00 (51.10)	4.00 (12.78)	4.00 (12.78)	4.00 (12.78)	4.00 (12.78)	4.00 (12.78)	2.00 (25.55)
	Hex	-	4.00 (5.08)	4.00 (5.08)	4.00 (5.08)	4.00 (5.08)	-	4.00 (5.08)	-	4.00 (5.08)
	DCM	-	-	4.00 (4.53)	-	-	-	-	-	-
	EtOAc	0.50 (172.40)	0.25 (172.40)	0.13 (331.54)	0.25 (172.40)	0.25 (172.40)	4.00 (10.78)	0.25 (172.40)	0.25 (172.40)	0.50 (86.20)
	MeOH	2.00 (155.42)	2.00 (155.42)	1.00 (310.83)	4.00 (77.71)	4.00 (77.71)	4.00 (77.71	4.00 (77.71)	4.00 (77.71)	2.00 (155.42)
P2 L	Dec	-	4.00 (11.15)	2.00 (22.30)	-	-	4.00 (11.15)	-	-	4.00 (11.15)
	Hex	4.00 (3.90)	4.00 (3.90)	-	2.00 (7.81)	4.00 (3.90)	4.00 (3.90)	4.00 (3.90)	4.00 (3.90)	-
	DCM	2.00 (5.21)	1.00 (10.42)	4.00 (2.61)	2.00 (5.21)	0.50 (20.84)	0.50 (20.84)	2.00 (5.21)	4.00 (2.61)	2.00 (5.21)
	EtOAc	1.00 (5.05)	0.50 (10.10)	0.50 (10.10)	2.00 (2.53)	2.00 (2.53)	1.00 (5.05)	4.00 (1.26)	1.00 (5.05)	1.00 (5.05)
	MeOH	2.00 (33.99)	1.00 (67.97)	1.00 (67.97)	2.00 (33.99)	4.00 (16.99)	-	4.00 (16.99)	2.00 (33.99)	2.00 (33.99)
P2 F	Dec	4.00 (14.80)	2.00 (29.60)	0.50 (118.40)	2.00 (29.60)	4.00 (14.80)	2.00 (29.60)	2.00 (29.60)	2.00 (29.60)	1.00 (59.20)
	Hex	4.00 (1.87)	2.00 (3.73)	0.13 (59.68)	2.00 (3.73)	4.00 (1.87)	4.00 (1.87)	4.00 (1.87)	4.00 (1.87)	0.25 (29.84)
	DCM	1.00 (42.3)	1.00 (42.3)	0.25 (169.2)	0.50 (84.60)	2.00 (21.15)	2.00 (21.15)	2.00 (21.15)	2.00 (21.15)	0.50 (84.60)
	EtOAc	1.00 (83.36)	0.50 (166.7)	0.25 (333.4)	0.50 (166.72)	1.00 (83.36)	2.00 (41.68)	1.00 (83.36)	1.00 (83.36)	0.50 (166.7)
	MeOH	2.00 (252.97)	2.00 (252.97)	0.50 (1011.86)	2.00 (252.97)	4.00 (126.4)	4.00 (126.4)	2.00 (252.9)	2.00 (252.9)	1.00 (505.9)
CIP*		0.00098	0.003	0.00078	0.063	0.25	0.0156	0.0078	0.0078	0.0031
CHL*		0.008	0.006	0.003	0.063	0.030	0.031	0.03	0.125	0.0078
STR*		0.008	0.008	0.008	1.00	0.063	0.008	0.02	0.50	0.25

P1: *Averrhoa bilimbi*; P2: *Phyllanthus acidus*; L: leaf; F: fruit; MIC: Minimum inhibitory concentration; DCM: dichloromethane; EtOAc: ethyl acetate; MeOH: methanol; clinical isolates: AC: *Acinetobacter spp*; EC*: Escherichia coli;* EF: *Enterococcus faecalis*; KL: *Klebsiella spp*; PR: *Proteus spp*; PM: *Proteus mirabilis*; SA: *Staphylococcus aureus;* MRSA: Methillin-Resistant *Staphyloccocus aureus*; *Positive control for bacteria; CHL: Chloramphenicol; CIP: Ciprofloxacin; STR: Streptomycin; ( ): total activity in ml/g; -: no activity; +: no bacterial growth at a stock concentration of 10 mg/ml; All data represents the mean of three independent experiments.

Table 3
Antioxidant and cytotoxicity of crude extracts.

Plants	Extract	DPPH, IC_{50} µg/ml (IC_{50} Range)	$ABTS^{\bullet+}$ [IC_{50} (range) µg/ml]	FRAP (TE µM crude extract)	Cytotoxicity (IC_{50} ± SD (µg/ml)
A. bilimbi Leaf (P1L)	Hex	-	-	153.37 ± 0.87	NT
	DCM	-	-	219.85 ± 2.17	NT
	EtOAc	91.41 (81.25–102.80)	-	192.89 ± 0.43	NT
	MeOH	34.85 (31.06–39.10)	39.26 (25.51–60.41)	87.04 ± 0.22	> 400
	Dec	5.30 (4.29–6.54)	31.68 (22.22–45.16)	109.86 ± 0.43	> 400
A. bilimbi Fruit (P1F)	Hex	-	-	25.92 ± 0.43	NT
	DCM	-	-	56.55 ± 0.43	NT
	EtOAc	-	-	71.57 ± 1.30	NT
	MeOH	79.09 (69.92–99.42)	-	22.55 ± 1.73	> 400
	Dec	-	-	26.23 ± 0.61	> 400
P. acidus Leaf (P2L)	Hex	-	-	120.89 ± 0.87	NT
	DCM	-	-	99.29 ± 0.65	NT
	EtOAc	-	-	75.55 ± 0.87	NT
	MeOH	148.6 (42.96–514)	-	78.61 ± 1.30	> 400
	Dec	19.15 (16.92–21.68)	-	59.83 ± 2.04	> 400
P. acidus Fruit (P2F)	Hex	-	-	ND	NT
	DCM	-	-	ND	NT
	EtOAc	-	-	90.71 ± 0.22	NT
	MeOH	198.4 (177.1–222.3)	-	23.98 ± 1.72	> 400
	Dec	-	-	81.99 ± 2.01	367.7 ± 22.34
Control		5.89 (4.96–6.99)[a] 0.96 (0.89–1.05)[b]	5.50 ± 0.02[a] 4.58 ± 0.05[b]	869.40 ± 43.81[a]	< 0.05[c]

P1: *Averrhoa bilimbi*; P2: *Phyllanthus acidus*; L: leaf; F: fruit; Hex: hexane; DCM: dichloromethane; EtOAc: ethyl acetate; MeOH: methanol; DPPH: 2,2-diphenyl-1-picrylhydrazyl radical; $ABTS^{\bullet+}$: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid radical cation; FRAP: Ferric reducing antioxidant power; TE: trolox equivalent. NT: not tested; Control: [a]ascorbic acid; [b]trolox; [c]Actinomycin D. All data represent the mean ± standard deviation of three independent analyses. IC_{50} (range) was calculated using GraphPad Prism 5.03. IC_{50} ± SD (µg/ml) < 50 µg/ml indicates high toxicity; IC_{50} ± SD (µg/ml) 50 < x < 100 µg/ml indicates moderate toxicity; IC_{50} ± SD (µg/ml) > 200 µg/ml indicates low toxicity; IC_{50} ± SD (µg/ml) > 1000 µg/ml indicates no toxicity.

Fakim and Gueho, 1996).

4. Conclusion

This study showed that extracts from *Phyllanthus* and *Averrhoa* species inhibited the growth of several human pathogenic bacteria. The findings from the present study indicated that the antimicrobial activity vary with the plant species and the nature of the extractive solvent. P1L decoction extract showed strong DPPH radical scavenging activity at IC_{50} of 5.30 µg/ml. Extracts from P1 and P2 indicated low activity against ABTS and FRAP assays. Methanol and decoction of leaf and fruit extracts of both P1 and P2 demonstrated low cytotoxicity. However, further toxicological studies should be conducted to assess their safety. Therefore, it can be suggested that leaf and fruits extracts from both species are potential source of natural antimicrobial agents. Further research is needed to isolate and identify bioactive compounds from the crude extracts.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.01.036.

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