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Antibiotic-potentiating activity, phytochemical profile, and cytotoxicity of *Acalypha integrifolia* Willd. (Euphorbiaceae)

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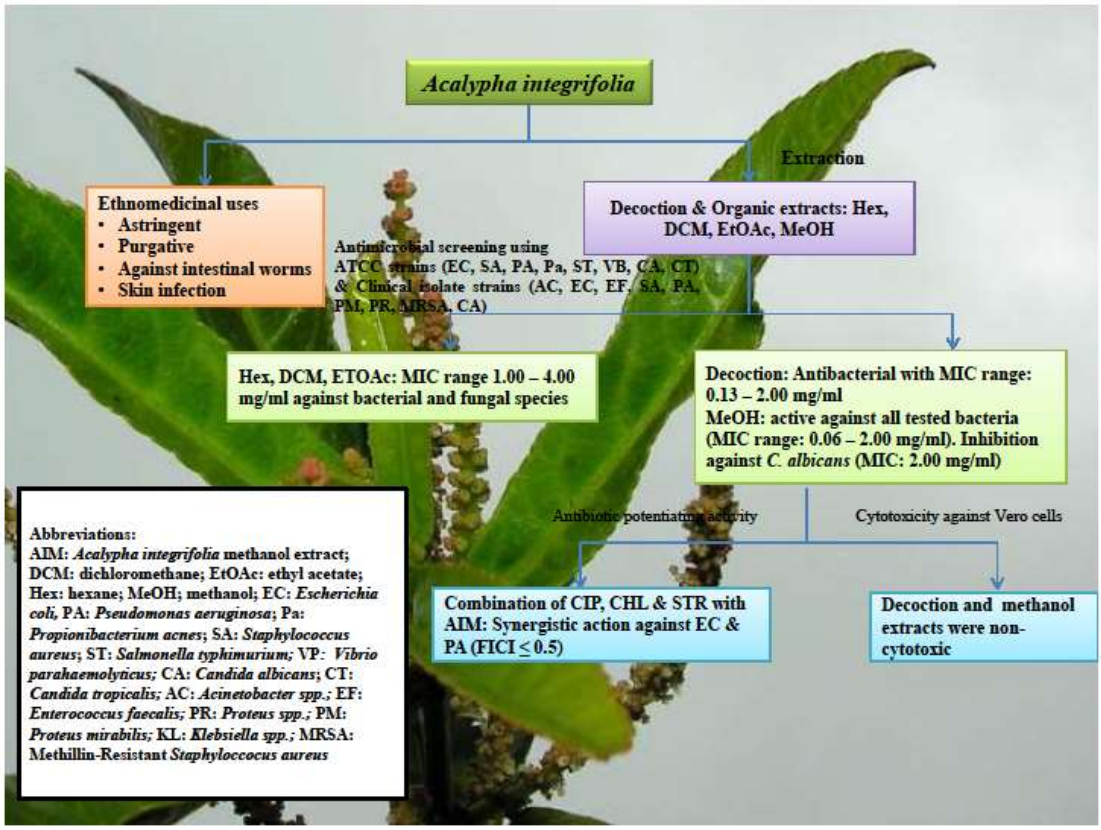
Graphical Abstract



Abstract

Acalypha integrifolia Willd. (Euphorbiaceae) (AI), an indigenous medicinal plant of the Mascarene Islands is traditionally used to manage infectious diseases. The authors aimed to evaluate the antimicrobial, antibiotic-potentiating activity and cytotoxicity of AI. Decoction as traditionally used and organic extracts (hexane, dichloromethane, ethyl acetate, and methanol) of AI leaves were screened for their antimicrobial activity against nine ATCC strains and 10

clinical isolates. A fractional inhibitory concentration index (FICI) was determined to establish any synergistic interaction between the extracts and antibiotics using the variable ratio analysis method. The phytochemical profile was established using chemical and thin-layer chromatography methods. The decoction and methanolic extracts inhibited the growth of all tested bacteria (minimum inhibitory concentration (MIC) ranged between 0.06-2.00 mg/ml). The methanolic extract showed potent antibacterial activity against *Escherichia coli* (EC) (MIC: 0.06 mg/ml), *Pseudomonas aeruginosa* (MIC: 0.50 mg/ml), and *Staphyloccocus aureus* (SA) (MIC: 0.06 mg/ml). The highest antibacterial activity was observed against clinical isolates of *Enterococcus faecalis, S. aureus*, and Methicillin-Resistant *S. aureus* (MRSA) (MIC: 0.50 mg/ml). Moderate antifungal activity was observed against *Candida albicans*. Combination profiles of the extract with Ciprofloxacin, Chloramphenicol, and Streptomycin showed synergistic action (FICI $\leq$ 0.50) against ATCC strains of EC and SA. Decoction and methanolic extracts showed low cytotoxicity against normal adult African green monkey kidney cells. Data obtained tends to corroborate with the reported traditional usage of the plant. Since the leaf decoction is traditionally used against skin infections as well as consumed for its astringent and purgative properties, the reported data tend to correlate with the low cytotoxicity as well as its anti-infective property. There may also be the potential towards the discovery of novel antimicrobial compounds.

Abbreviations: AI: *A. integrifolia*, Chlo: chloroform, MeOH: methanol, DCM: dichloromethane, EtOAc: ethyl acetate, EC: *Escherichia coli*, SA: *Staphylococcus aureus,* ST: *Salmonella typhimurium*, VP: *Vibrio parahaemolyticus,* AC: *Acinetobacter spp.*, KL: *Klebsiella spp.*, PA *Pseudomonas aeruginosa*, EF: *Enterococcus faecalis*, PR: *Proteus spp.*, CA: *Candida albicans*, CT: *Candida tropicalis*, NT: not tested, CHL: chloramphenicol, CIP: ciprofloxacin, GEN: gentamicin, STR: streptomycin, AM B: amphotericin B, NYS: nystatin, NA: not available, MIC: minimum inhibitory concentration, FIC: fractional inhibitory concentration, FICI: fractional inhibitory concentration index.

Keywords: *Acalypha integrifolia*; Ethnomedicinal; antimicrobial; antibiotic potentiating; cytotoxicity; Mascarene Islands.

1.0. Introduction

Antimicrobial resistance is a worldwide threat and has huge economic implications (WHO, 2015). Multidrug-resistant bacteria in the European Union are estimated to cause an economic loss of more than 1.5 billion euros each year and international organisations are increasingly reporting the risks of antimicrobial resistance to global health security (WHO, 2015, Mundy et

al., 2016). In the United States, at least two million people are infected each year with bacteria that are resistant to antibiotics and at least 23000 deaths are recorded each year as a result of these infections (CDC, 2015). Few antibiotics are being approved by regulatory organizations, which reflects both the difficulty of developing such agents and the fact that antibiotic discovery programs have been terminated at several major pharmaceutical companies in the past decade (Cottarel and Wierzbowski, 2007, Mundy et al., 2016). According to Eloff (2015), 25% of the prescribed pharmaceuticals for indications other than antibiotics are of plant origin, while the majority of antibiotics originate from fungal leads, there has been no development of commercial antibiotics from plant origin.

Mauritius has a very rich and diverse flora and many native, indigenous, and endemic plants that are used in folk medicine for the treatment of various diseases (Chintamunnee and Mahomoodally, 2012). However, few medicinal plant species of Mauritius have been scientifically evaluated for their possible medicinal properties. A recent review on the *Acalypha* genus revealed that there is no published data on the *in vitro* and *in vivo* evaluation of *Acalypha integrifolia* species (Seebaluck et al., 2015). *Acalypha integrifolia* occurs in Madagascar, Réunion Island, and Mauritius (Schmelzer, 2007). In Réunion Island and Mauritius, leaf decoctions are consumed as an astringent and purgative and also used to eliminate intestinal worms. Bathing in leaf decoctions is effective for the treatment of skin infections (Gurib-Fakim and Gueho, 1996, Schmelzer, 2007, Seebaluck et al., 2015). Furthermore, species from the *Acalypha* genus have been reported worldwide as traditional remedies against an array of ailments which include fever, dysentery, jaundice, liver inflammation, diabetes, hypertension, respiratory problems including bronchitis, asthma, pneumonia as well as skin conditions such as scabies, eczema, and mycoses (Seebaluck et al., 2015). Nonetheless, there is still a dearth of scientific validation of the traditional claims of this species. Thus, the present study was designed to assess the *in vitro* antimicrobial and antibiotic potentiating effects of *A. integrifolia* extracts with a view of finding alternate and promising sources of antimicrobial agents.

2. Materials and methods

2.1. Preparation of plant materials and extraction

Fresh leaves of *Acalypha integrifolia* Willd. subsp. *integrifolia var. integrifolia* were obtained from Monvert Nature Park, Mauritius (latitude 20° 33' 26S, longitude 57° 53' 54E). The plant was identified by Mr. Kersley Pynee, botanist at the Mauritius Herbarium, Agricultural Services (Barcode number: MAU 0019766). The leaves were dried at 40°C in a drying cabinet

for 4 to 5 days until constant mass was obtained. The decoction as traditionally used was tested together with organic extracts for comparative purpose. For the preparation of the decoction, 50g of dried plant powder was extracted with 200 ml of distilled water at 100ºC until the volume decreased to 1/4 of the original volume. It was then filtered through layers of muslin cloth and Whatman number 1 filter paper and then dried under reduced pressure using a rotary evaporator. Organic exhaustive extraction of the dried powdered sample was carried out *via* serial maceration using hexane, dichloromethane (DCM), ethyl acetate, and methanol (10:1 solvent to dry weight ratio). The extracts were filtered, combined, and dried under reduced pressure using a rotatory evaporator (Rangasamy et al., 2007).

2.2. Qualitative phytochemical screening

The phytochemical profiles of the extracts were determined using standard protocols as described by Ben et al., (2013), Kumar et al., (2013), Narendra et al., (2013), Sathya et al., (2013), Solomon et al., (2013), and Philip et al., (2011). Some phytochemical classes such as cardiac glycosides, coumarins, terpenes, sesquiterpenes, flavonoids, alkaloids, sugars, and tannins were evaluated *via* thin layer chromatography using standard protocol (Wagner and Bladt, 1996).

2.3. Antimicrobial assay

2.3.1. Microdilution broth susceptibility assay

A two-fold serial microdilution technique was used to evaluate the minimum inhibitory concentration values for the different extracts as described by Shai et al., (2008). The microorganisms used in this study included reference strains from the 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), *Candida albicans* (ATCC 10231), *Candida tropicalis* (ATCC 750) and clinical laboratory isolates such as *Escherichia coli*, *Staphylococcus aureus*, *Acinetobacter spp.*, *Klebsiella spp.*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Proteus spp.*, *Proteus mirabilis*, Methicillin-Resistant *Staphyloccocus aureus* (MRSA), and *Candida albicans*. The clinical isolates were obtained from Victoria Hospital, Candos, Mauritius.

One hundred microlitres of each extract (16 mg/ml) was serially diluted two-fold in triplicate with sterile peptone water in 96-well microtitre plates. Fresh bacterial and fungal inoculums were prepared and adjusted to 0.5 McFarland standard which were further diluted at a ratio of

1:100 with fresh sterile peptone water broth to yield starting inoculums of approximately 10^6 CFU/ml. One hundred microliters of bacterial or fungal culture was added to each well of bacterial and fungal plates respectively. Chloramphenicol, Ciprofloxacin, Gentamicin, and Streptomycin were used as positive controls for the anti-bacterial while Nystatin and Amphotericin B were used for the anti-fungal assay. Negative controls included sterile peptone water broth. The bacterial and fungal plates were incubated at 37 ºC for 24 h and 48 h respectively. After incubation, 40 µl of iodo-nitrotetrazolium chloride (INT: 0.2 mg/ml) was added to each well and the plates further incubated for 20 min. Bacterial and fungal growth was denoted by red colouration. The well of lowest concentration in which no pinkish red colouration was observed was considered to be the 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) (Shai et al., 2008).

2.3.2. Antibiotic potentiating assay

The methanolic extract of *A. integrifolia* gave the lowest MIC values against most of the tested bacteria and thus was investigated in conjunction with commercial antibiotics namely Ciprofloxacin (CIP), Chloramphenicol (CHL), and Streptomycin (STR) to determine any possible synergistic activity using variable ratio analysis method (De Rapper et al., 2012). Three different ratios of extract:antibiotic were prepared (30:70, 50:50, and 70:30) by mixing known volume of stock solution of the extract (16 mg/ml) with CIP (0.1 mg/ml), CHL (1 mg/ml), and STR (1 mg/ml) respectively. The assay was carried out *via* micro-dilution method as described in section 2.3.1. One hundred microlitres of each extract: antibiotic (30:70, 50:50 and 70:30) combination were serially diluted two-fold with sterile peptone water, in triplicate in a 96-well microplate for each of the ATCC bacterial strains. The different antibiotics (CIP, CHL, and STR) were used alone as positive controls and sterile peptone water as negative control. Inoculum (100 µl) was added to each well. The plates were then incubated overnight at 37 ºC. After incubation, 40 µl of INT (0.2 mg/ml) was added to each well and the plates were further incubated for 20 min at 37ºC. The MICs were recorded and the results of the combined effects of the antibiotics and extracts were calculated and expressed in terms of a fractional inhibitory concentration index (FICI) which is denoted by the following formula:

$FIC_{extract}$ = MIC of extract in combination/MIC of extract alone

$FIC_{antibiotic}$= MIC of antibiotic in combination/MIC of antibiotic alone

$FICI = FIC_{extract} + FIC_{antiobiotic,}$

$FIC_{extract}$ is the fractional inhibitory concentration of the extract and $FIC_{antiobiotic}$ is the fractional inhibitory concentration of the antibiotic used.

2.4. Cell culture

The Normal adult African green monkey kidney (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 foetal bovine serum, FBS). The cells were grown in a humidified incubator set at 5% CO_2 and 37 ˚C. After the formation of a confluent monolayer, the cells were sub-cultured. The cells were detached by treating them with trypsin-EDTA (0.25% trypsin containing 0.01% EDTA) for 10 minutes followed by the addition of supplemented media to inhibit the reaction.

2.5 *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 Cell Proliferation Kit II and method described by Zheng et al. 2001. Briefly, 100 µl of Vero cells were seeded in a 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. Extracts were prepared to a stock solution of 20 mg/ml, added to the microtitre plate and serially diluted to range from 400 µg/ml-1.563 µg/ml. 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 multiwell plate reader. The assay was performed in triplicate to calculate an IC_{50} of the cell population for the decoction and methanolic extracts.

2.6. Data analysis

All determinations were carried out in triplicate. Calculation of IC_{50} was carried out using GraphPad Prism Version 5.03 for Windows (GraphPad Software Inc.).

Results and discussion

3.1. Antibacterial activity

The results of the antibacterial activity of AI extracts against the tested ATCC strains are illustrated in Table 1. The antimicrobial activity of a crude extract can be considered significant if its MIC value is lower than 1 mg/ml (Pendota et al., 2015). Thus, in this study, MIC values lower than 1 mg/ml were considered as significant. The MIC ranged between 0.06 to 4.00 mg/ml. The hexane, dichloromethane, and ethyl acetate extracts showed moderate activity at MIC range of 1.00 to 4.00 mg/ml. The decoction showed significant activity against *S. aureus* (MIC: 0.25 mg/ml). The extract revealed noteworthy inhibitory activity against ATCC strain of *P. aeruginosa* at MIC value of 0.25 mg/ml when compared to the positive control Streptomycin. The methanolic extract gave the lowest MIC value (0.06 mg/ml) against ATCC strains of *E. coli* and *S. aureus*. Significant activity was also observed against *Salmonella typhimurium* and *Vibrio parahaemolyticus* with MIC values 0.25 and 0.50 mg/ml respectively. Both the decoction and methanolic extracts showed inhibitory activity against *P. acnes* - ATCC 11827 and 6919 (MIC range: 0.13-0.50 mg/ml), while also showing inhibition against all of the tested clinical isolates. The decoction displayed remarkable inhibitory activity against *Acinetobacter spp.* (MIC: 0.25 mg/ml) which was higher than the positive control Streptomycin (MIC: 1.00 mg/ml). Notable activity was recorded against *S. aureus* (MIC: 0.13 mg/ml), *P. mirabilis*, *Proteus*, and *Klebsiella* species (MIC values: 0.50 mg/ml). The methanolic extract demonstrated lowest MIC value (0.50 mg/ml) against *E. faecalis*, *S. aureus*, and Methicillin-Resistant *S. aureus* (MRSA). Bacteria such as *Enterococcus faecalis* can cause endocarditis, bladder, prostate gland, and epididymal infections (Orhan et al., 2010). *Acinetobacter* species are responsible for serious infectious diseases such as ventilator associated pneumonia, bacteraemia, urinary tract infections, burn wound infections, endocarditis, secondary meningitis, and septicaemia, involving mostly patients with impaired host defences, especially in intensive care units (Swe-Han and Pillay, 2015). *Proteus mirabilis* is related to wound infections and urinary tract infections in the elderly and young males often following catheterisation or cystoscopy and is also a secondary invader of ulcers (Pandey and Kumar, 2013). MRSA is linked with skin infections as well as life-threatening bloodstream infections, pneumonia, and surgical site infections (CDC, 2015). Thus, the decoction and methanolic extracts can be considered a potential broad spectrum antimicrobial agent for the management of numerous severe infections.

The total activity values indicated that the methanolic extract exhibited the highest antibacterial activity. The antibacterial activity of AI extracts was in the order of methanol > decoction > dichloromethane, and ethyl acetate > hexane. The findings from the present study indicated that the antimicrobial activity may vary with the nature of the extraction solvent (Kanyanga et al., 2014). The ethnomedicinal uses of the plant are related to infections. A bath with the plant decoction is used to manage skin diseases. The plant is an astringent and depurative and is used against eczema (Gurib-Fakim and Gueho, 1996). The results of this study confirm the reported traditional use of the leaf decoction against infectious conditions. The hexane extract showed the absence of bioactive compounds such as tannins, polyphenols, and coumarins that could account for the low activity. All extracts of *A. integrifolia* showed the presence of terpenes, cardiac glycosides, and sugars (Tables 3, 4, and 5). The presence of different phytochemicals such as tannins, phenols, terpenes, and coumarins could be related to the broad spectrum activity of the decoction and methanolic extracts.

3.2. Antifungal activity

The lowest MIC values (1.00 and 0.50 mg/ml) were observed with the ethyl acetate extract against ATCC strains of *C. albicans* and *C. tropicalis* after 48 h (Table 1). The dichloromethane extract was active against ATCC strains of *C. albicans* and *C. tropicalis* while the decoction was inactive against both species. However, the total antifungal activity indicated that methanolic extract was the most active. The methanolic extract showed antifungal activity against clinical isolate strain of *C. albicans* (MIC: 2.00 mg/ml) which was lower than the positive control Nystatin (MIC: 4.00 mg/ml). It also showed more potent activity than Amphotericin B, which was inactive against clinical isolate of *C. albicans*. The diversity of secondary metabolites such as terpenes, polyphenols, tannins, coumarins, and flavonoids as well as the extraction solvent could be responsible for the bioactivity of the extracts (Martins et al., 2015).

3.3. Antibiotic potentiating activity

Synergy can be described as the effect of a combination of substances which is greater than would be expected by adding together their separate contributions (Mundy et al., 2016). In this study, *A. integrifolia* methanolic (AIM) extract showed significant antibacterial activity and thus was further tested in combination with antibiotics namely CIP, CHL, and STR at different ratios. The results of the combined effects of the antibiotics and extracts were expressed in terms of a fractional inhibitory concentration index (FICI) which is equal to the sum of the

FICs of the antibiotic and extract (Table 2). The results were considered synergistic if the FICI of the combination is ≤0.5, additive when it was 0.5 <FICI <1, subtractive when FICI is greater than 1 and less than 4, or antagonism for FICI > 4 (De Rapper et al., 2012, Duarte et al. 2012, Chaudhary et al., 2013).

When combined with Ciprofloxacin (30% and 50%), AIM showed synergistic activity against *E. coli* (FICI: 0.16 and 0.38 respectively). Synergy was also observed with AIM in combination with 50% Ciprofloxacin when tested against *S. aureus* (FICI: 0.26). All combinations of Chloramphenicol and Streptomycin with AIM indicated synergistic behaviour against *S. aureus* (FICI ≤ 0.5). Mixing AIM with Streptomycin showed synergism against *E. coli* with FICI ranging from 0.31 to 0.48.

AIM combined with Ciprofloxacin at 30% and 50% showed a shift towards additive (FICI 0.74 and 0.68 respectively), however showed subtractive behaviour (FICI: 1.38) at a high concentration of Ciprofloxacin (70%) against *P. aeroginosa*. Conversely, when Chloramphenicol was used at a low concentration (30%), the effect was subtractive (FICI: 1.70), while increasing the concentration to 70% showed additive activity (FICI: 0.65). All concentrations of AIM combined with Streptomycin displayed a subtractive behaviour (FICI range: 1.13 to 1.55) against *P. aeroginosa*.

The presence of one or more groups of secondary metabolites such as tannins, polyphenols, coumarins, flavonoids, saponins, and glycosides are responsible for antimicrobial activity alone or in combination (Ahmad and Aqil, 2007). The modes of action of commercial antibiotics are well known. Chloramphenicol and its metabolites act primarily on the 50S ribosomal subunit which suppresses the activity of the enzyme peptidyltransferase. It inhibits

mitochondrial membrane protein synthesis, leading to suppression of mitochondrial respiration and ultimately cessation of cell proliferation (IEADRI, 2016). Streptomycin binds to the 16S rRNA of the bacterial ribosome and blocks protein synthesis (Grosset and Singer, 2013). Moreover, Ciprofloxacin inhibits DNA synthesis by promoting cleavage of bacterial DNA in the DNA-enzyme complexes of DNA gyrase and type IV topoisomerase, causing rapid bacterial death (Oliphant, 2002). The exact mechanism of antimicrobial activity of plant compounds is not clearly defined. However, multiple dose-dependent pathways have been postulated. These include damage of membrane at bacteriocidal concentrations of plant compounds as well as loss of energy production which finally lead to cell death (Upadhyay et al., 2015). Studies indicated that sub-lethal or sub-inhibitory concentrations of plant compounds affect virulence in Gram-positive and Gram-negative bacteria as well as fungal pathogens by modulating gene transcription, protein expression and quorum sensing (Upadhyay et al., 2015). Moreover, *E. coli* is linked with septicemia and meninges, surgical wounds, skin lesions and can infect the gall bladder. *Pseudomonas* strains are responsible for neonatal nosocomial infections (Pandey and Kumar, 2013). *Staphylococcus aureus* can lead to skin and soft tissue infections as well as other severe infections such as bacteremia, pneumonia, acute endocarditis, meningitis, osteomyelitis, toxic shock syndrome, and fatal invasive diseases (Martinez et al., 2014). Therefore, the combination therapy involving Chloramphenicol, Ciprofloxacin, Streptomycin, and *A. integrifolia* extract represent a feasible strategy to manage various infectious diseases.

3.4. Cytotoxicity

Cytotoxicity evaluation is important to validate the safety of medicinal plants for traditional usage (Latif et al., 2014). The cytotoxic effect of the decoction and methanolic extracts were investigated using normal adult African green monkey kidney cells (Vero cells). Actinomycin D was used as the positive control. The IC_{50} values for the decoction and methanol extracts were >400 µg/ml and 205.4±3.81 µg/ml respectively, while that of the positive control was <0.05 µg/ml. According to Kuete and Efferth (2015), the threshold values for defining cytotoxicity while dealing with edible parts of plants, culinary plants, and spices, are as follows: significant or strong cytotoxicity: IC_{50} < 50 µg/ml; moderate cytotoxicity: 50 µg/ml < IC_{50} < 200 µg/ml; low cytotoxicity: 200 µg/ml < IC_{50} < 1000 µg/ml; no cytotoxicity: IC_{50} > 1000 µg/ml'. Thus, both decoction and methanol extracts showed low cytotoxicity compared to the

positive control. This study tends to justify the local claims on the efficacy of the leaf decoction against infectious conditions. Furthermore, the methanolic extract can be further explored and considered in the management of bacterial and fungal infections. However, further *in vitro* as well as *in vivo* toxicological studies should be conducted to evaluate its safety.

3.5. Phytochemical screening

The phytochemicals of the different extracts were determined by qualitative colour tests and TLC bioautography, the results of which are summarised in Tables 3 and 4. The R_f values of the different phytochemicals are illustrated in Table 5. All extracts showed the presence of terpenes (R_f values: 0.2-0.98) and cardiac glycosides (R_f values: 0.14-0.97). The methanolic extract showed the possible presence of saponins and anthocyanins. Most extracts showed the presence of flavonoids. Various flavonoid derivatives have been reported to possess antimicrobial potential against different microorganisms. Flavonoid derivatives, namely 6,8-diprenyleriodictyol, isobavachalcone, 6-prenylapigenin and 4-hydroxylonchocarpin, were found to depolarize the membrane of *S. aureus* and inhibit DNA, RNA as well as protein synthesis (Dzoyem et al., 2013). The decoction, ethyl acetate, and methanolic extracts showed the presence of a wide range of phytochemicals including coumarins (R_f values: 0.19-0.96), tannins (R_f values: 0.03-0.13), and sesquiterpene lactones (R_f values: 0.33-0.93). Tannins have been shown to inactivate microbial adhesins, enzymes, and cell envelope transport proteins which could explain, to some extent, the antimicrobial activity of these extracts (Ciocan and Bara, 2007). Alkaloids, anthraquinones, steroids, terpenoids, and quinones were found to be absent in all extracts.

4.0. Conclusion

Results from this study showed that extracts from *Acalypha integrifolia* inhibited the growth of several human pathogenic bacteria and fungus. The ability of the extracts to suppress the growth of the investigated pathogens further validates the folkloric uses of this plant against infectious conditions. The authors found that the decoction inhibited the growth of all tested bacteria and was also found non-toxic. The synergistic effect of *A. integrifolia* methanolic extract in combination with commercial antibiotics could lead to new developments against

antimicrobial resistance for the management of infections. Moreover, both the decoction and methanolic extracts showed low cytotoxicity against normal adult African green monkey kidney cells. The broad spectrum antimicrobial potential of *A. integrifolia* extracts could therefore make this species a candidate for antibiotic bioprospecting. Nevertheless, further pharmacological and *in vivo* toxicological studies should be conducted to assess its safety.

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Table 1: Antimicrobial activity of crude extracts

	MIC (mg/ml)																	
	ATCC strains								Clinical isolates									
Extracts	**EC**	**PA**	**Pa[1]**	**Pa[2]**	**SA**	**ST**	**VB**	**CA**	**PM**	**MRSA**	**EF**	**PA**	**SA**	**EC**	**AC**	**KL**	**PR**	**CA**	**CT**
Hexane	-	-	NT	NT	4.00 (3.00)	2.00 (6.00)	1.00 (12.00)	-	4.00 (3.00)	2.00 (6.00)	2.00 (6.00)	4.00 (3.00)	4.00 (3.00)	4.00 (3.00)	2.00 (6.00)	2.00 (6.00)	4.00 (3.00)	-	1.00 (12.00)
DCM	2.00 (3.00)	4.00 (1.00)	NT	NT	2.00 (3.00)	4.00 (1.00)	4.00 (1.00)	2.00 (3.00)	2.00 (3.00)	2.00 (3.00)	2.00 (3.00)	4.00 (1.00)	4.00 (1.00)	4.00 (1.00)	2.00 (3.00)	2.00 (3.00)	2.00 (3.00)	NT	1.00 (5.00)
EtOAc	2.00 (3.00)	4.00 (1.00)	NT	NT	2.00 (3.00)	4.00 (1.00)	4.00 (1.00)	1.00 (5.00)	-	2.00 (3.00)	-	2.00 (3.00)	1.00 (6.00)	4.00 (1.00)	2.00 (3.00)	4.00 (1.00)	-	NT	0.50 (11.00)
MeOH	0.06 (1729)	0.50 (218)	0.13 (872)	0.13 (872)	0.06 (1729)	0.25 (436)	0.50 (218)	2.00 (54.5)	2.00 (54.5)	0.50 (218)	0.50 (218)	2.00 (54.5)	0.50 (218)	1.00 (108)	2.00 (54.5)	1.00 (108)	1.00 (108)	2.00 (54.5)	-
Decoction	1.00 (134.3)	0.25 (537.3)	0.50 (268.6)	0.50 (286.6)	0.25 (537.3)	1.00 (134.3)	2.00 (67.2)	-	0.50 (268.6)	1.00 (134.3)	1.00 (134.3)	2.00 (67.2)	0.13 (1074.5)	2.00 (67.2)	0.25 (537.3)	0.50 (268.6)	0.50 (268.6)	-	-
Control																			
CIP*	0.0004	0.025	NT	NT	0.0008	0.0008	0.0002	NT	0.0078	0.0031	0.0156	0.003	0.00078	0.00098	0.063	0.25	0.0078	NT	NT
CHL*	0.0039	0.0625	NT	NT	0.0031	0.0078	0.0039	NT	0.125	0.0078	0.031	0.006	0.003	0.008	0.063	0.030	0.03	NT	NT
STR*	0.0156	0.25	NT	NT	0.0039	+	+	NT	0.50	0.25	0.0080	1.00	0.008	0.008	1.00	0.063	0.02	NT	NT
TET*	NT	NT	0.0016	0.0008	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
NYS**	NT	NT	NT	NT	NT	NT	NT	0.016	NT	NT	NT	NT	NT	NT	NT	NT	NT	4.00	0.002
AMB**	NT	NT	NT	NT	NT	NT	NT	0.063	NT	NT	NT	NT	NT	NT	NT	NT	NT	-	0.031

MIC: Minimum inhibitory concentration; DCM: dichloromethane; EtOAc: ethyl acetate; MeOH: methanol; ATCC strains: CA: *Candida albicans* ATCC 10231; 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; 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*; CA: *Candida albicans*; CT: *Candida tropicalis* ATCC 750; *Positive control for bacteria; **: Positive control for fungus; CHL: Chloramphenicol; CIP: Ciprofloxacin; GEN: Gentamicin; STR: Streptomycin; TET: tetracycline; AMB: Amphotericin B; NYS: Nystatin. ( ): 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.

Table 2: FIC and FICI of *A. integrifolia* methanolic extract in combination with conventional antibiotics

Extract [%] Control (%)	*E. coli*		*P. aeruginosa*		*S. aureus*	
	FIC	**FICI**	**FIC**	**FICI**	**FIC**	**FICI**
AIM [70]	0.09	0.16*	0.70	0.74	0.35	0.66
CIP (30)	0.08		0.04		0.31	
AIM [50]	0.13	0.38	0.50	0.68	0.25	0.26
CIP (50)	0.25		0.18		0.01	
AIM [30]	0.15	0.85	1.200	1.38	0.30	1.00
CIP (70)	0.70		0.18		0.70	
AIM [70]	0.09	0.39	1.40	1.70	0.18	0.33
CHL (30)	0.30		0.30		0.15	
AIM [50]	0.06	0.56	0.50	0.75	0.13	0.38
CHL [50]	0.50		0.25		0.25	
AIM (30)	0.04	0.74	0.30	0.65	0.08	0.43
CHL (70)	0.70		0.35		0.35	
AIM (70)	0.18	0.48	1.40	1.48	0.35	0.50
STR (30)	0.30		0.08		0.15	
AIM (50)	0.06	0.31	1.00	1.13	0.25	0.50
STR (50)	0.25		0.13		0.25	
AIM (30)	0.04	0.39	1.20	1.55	0.15	0.50
STR (70)	0.35		0.35		0.35	

FIC: fractional inhibitory concentration; FICI: fractional inhibitory concentration index; AIM: *A. integrifolia* methanol extract; CIP: ciprofloxacin; CHL: chloramphenicol; STR: streptomycin; *E. coli*: *Escherichia coli* ATCC 25922; *P. aeruginosa*: *Pseudomonas aeruginosa* ATCC 27853*; S. aureus: Staphylococcus aureus* ATCC 25923. *underline value indicate synergistic activity.

Table 3: Qualitative phytochemical analysis using chemical reactions

Extract	Terpenes	Tannins, polyphenols	Saponins	Antho-cyanins	Coumarins	Alkaloids	Flavonoids	Anthra-quinones	Sugars	Steroids	Terpenoids	Quinones
Hexane	+	-	-	-	-	-	+	-	+	-	-	-
DCM	+	-	-	-	-	-	+	-	+	-	-	-
EtOAc	+	+	-	-	+	-	+	-	+	-	-	-
MeOH	+	+	Possible +	Possible +	+	-	+	-	+	-	-	-

DCM: dichloromethane; EtOAc: ethyl acetate; MeOH: methanol; +: present, -: not detected.

Table 4: Phytochemical analysis *via* TLC

Extracts	Terpenes	Tannins, polyphenol	Coumarins	Alkaloids	Flavonoids	Sesquiterpene lactones	Sugars	Cardiac glycosides
Hexane	+	-	-	-	+	-	+	+
DCM	+	-	-	-	+	-	+	+
EtOAc	+	+	+	-	+	+	+	+
MeOH	+	+	+	-	+	+	+	+
Decoction	+	+	+	-	-	+	+	+

DCM: dichloromethane; EtOAc: ethyl acetate; MeOH: methanol; +: present, -: not detected

Table 5: R_f values of the different phytochemical classes

Extracts	Solvent system	Phytochemical	R_f values
Hex	Hex:EtOAc (2:1)	Terpenes	0.72, 0.83, 0.93, 0.97
DCM			0.77, 0.95
EtOAc			0.2, 0.43, 0.52, 0.77, 0.95
MeOH	MeOH:Chlo:Hex (30:40:30)		0.34, 0.44, 0.98
Decoction			0.32, 0.45
Hex	Hex:EtOAc (2:1)	Cardiac glycosides	0.97
DCM			0.95
EtOAc	MeOH:Chlo:Hex (30:40:30)		0.29, 0.30, 0.66
MeOH			0.14, 0.18, 0.44
Decoction			0.15, 0.25, 0.44
Hex	Hex:EtOAc (2:1)	Tannins	-
DCM			-
EtOAc			0.03, 0.06, 0.13
MeOH			0.04
Decoction			0.04
Hex	Hex:EtOAc (5:5)	Coumarins	-
DCM			-
EtOAc	DCM:EtOAc: MeOH (40:40:30)		0.19, 0.48, 0.53, 0.92
MeOH	MeOH:Chlo:Hex (30:40:30)		0.96
Decoction			0.75
Hex	Hex:EtOAc (5:5)	Flavonoids	0.64, 0.76, 0.95
DCM			0.99, 0.73, 0.83
EtOAc			0.99
MeOH			0.99
Decoction			-
Hex	Hex:EtOAc (5:5)	Sesquiterpene lactone	-
DCM			-
EtOAc	DCM:EtOAc:MeOH (40:40:30)		0.33, 0.80, 0.93
MeOH			0.51
Decoction			0.54

Chlo: chloroform; Hex: hexane; DCM: dichloromethane; EtOAc: ethyl acetate; MeOH: methanol; -: not detected;