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Antimicrobial and Antioxidant Activities of Crude Methanol Extract and Fractions of *Andrographis paniculata leaf* (Family: Acanthaceae) *(Burm. f.) Wall. Ex Nees*

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Abstract

Assessment of the antimicrobial and antioxidant activities of different solvent fractions of crude methanol extract of *Andrographis paniculata* leaf was carried out to investigate their medicinal properties. The test samples, crude methanol eextractt and its n-hexane, ethyl acetate, chloroform and water soluble fractions were tested against five clinical isolates: *Enterobacter cloacae, Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Candida albicans.* All the test samples showed antimicrobial activity against the test organisms, except for *Escherichia coli* which showed resistance to all the samples at the studied concentrations and *Candida albicans* which showed resistance to all the samples except for ethyl acetate with diameter zones of inhibition ranging from 11.5-17.5 mm and water soluble fractions with diameter zones of inhibition ranging from 11.5-13.0 mm; both in concentration dependent manner. The highest flavonoid content (41.79±0.44 µg QE/mg) and phenolic content (26.79±0.66 µg GAE/mg) were shown by the crude methanol extract and the n-hexane fraction, respectively while the chloroform fraction showed the least concentrations for both flavonoid (11.66±0.12 µg QE/mg) and phenolic (16.17±0.61 µg GAE/mg). *In vitro* antioxidant study using 2, 2-diphenyl-1-picrylhydrazyl scavenging assay showed that the crude methanol extract had the highest free radical scavenging activity with average percentage inhibition of 54.50±0.10 while the hexane fraction showed the least with average percentage inhibition of 11.36±0.10. Conclusively, the various solvent fractions of crude methanol extract of *Andrographis paniculata*; especially the ethyl acetate fraction could be considered a remedy for various infections and diseases which are associated with both the test organisms and free radicals.

Key words: *Andrographis paniculata,* Fractions, Antimicrobial, Antioxidant, Phenolic, Flavonoid.

1. Introduction

Andrographis paniculata Nees, commonly called "King of Bitters or Creat or Green Chirayta,'' is an important medicinal plant which belongs to the family Acanthaceae. It is a renowned annual traditional herbaceous crop with immense therapeutic properties (Datta *et al*., 2012) and it is widely cultivated and used in South Asia, India and China. In Ayurvedic formulations, it is one of the most extensively used plants (Okeke *et al*., 2001). It is a hardy and erect herb which grows mainly as an under-shrub in tropical, moist deciduous forest. It has glabrous leaves, about 8.0cm long and 2.5cm broad and white flowers with rose- purple spots on the petals (Nirlep, 2016). The stem is dark green, about 0.3- 1.0m in height and 2-6mm in diameter (Zhang, 2004; Niranjan *et al*., 2010). Some of its vernacular names include; Chuan xin Lian (Chinese), Kalmegh (Urdu), Kirayat (Hindu), Aluy (Philippines), Andrograhis (Spanish/Russian), Senshinren (Japenese) and India echinacea (Indian) (Jarukamjorn and Nemoto, 2008; Mishra *et al*., 2007; Sharma and Sharma, 2013). It is known as *Bhui-neem*, meaning "neem of the ground," since the plant, though being a small annual herb, has a similar strong bitter taste as that of the large Neem tree (Neha, 2016) and in Malaysia, it is known as *Hempedu Bumi*, which literally means 'bile of earth' since it is one of the most bitter plants which are used in traditional medicine. It is sometimes locally referred to as 'Ewe Jogbo' (Jogbo leaf) because of its bitterness but popularly called 'Mejemeje' (seven-seven) among 'Yoruba' speaking natives in Nigeria because an average dosage comprises of seven leaves eaten raw once or twice daily for about five days in the treatment of febrile illness or chronic debility and in the treatment of hypertension (Dada-Adegbola *et al*., 2014). The whole part of *A. paniculata* as well as its roots and aerial parts have been found useful for medicines over the years (Agbolahor *et*

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al., 2014) although (Aniel *et al*., 2010) have stated that the parts of the plant mostly used for medicinal purpose are the leaves and roots. Some of the chemical constituents that have been found in *A. paniculata* are: Diterpenes, flavonoids, iterpenoid, lactones, alkanes, alkaloids, glycoside, tannins, saponins ketones, aldehydes, paniculides, farnesols, polyphenols, arabinogalactan, and several sub-units of andrographolides (Niranjan *et al*., 2010; Akbar, 2011; Sharma and Joshi, 2011).

A. paniculata has been reportedly used for many years to successfully combat various diseases, such as skin infections, herpes, dysentery, fever, sore throat, lower urinary tract infections, gastrointestinal tract and upper respiratory tract infections, inflammation, diarrhea, pneumonia, tonsillitis, gastroenteritis, pyelonephritis and laryngitis (Shalini and Narayanan, 2015; Wangboonskule *et al*., 2006; Mishra *et al*., 2007; Sharma M and Joshi, 2011; Dhiman *et al*., 2012). It has been used as an immune system booster and for the treatment of many chronic infections (Nirlep, 2016; Chandrasekaran *et al*., 2009). Its hepatoprotective effect has also been reported (Abdulaziz *et al*., 2014). It has been reported as antidote for snakebite and poisonous insects (Dhiman *et al*., 2012; Samy *et al*., 2008), anti-diabetes and anti-malarial agent (Agarwal *et al*., 2009; Mishra *et al*., 2009). However, some of its adverse effects may include gastric instability, loss of appetite, diarrhea, metallic taste and allergic reactions; it is not recommended for pregnant women (Sachin and Kailasam, 2017). Various solvent extracts of *A. paniculata* have also been reported for a wide spectrum of pharmacological activities which include antiviral, choleretic, hypoglycemic, hypocholesterolemic, anti-microbial, antioxidant, anti-inflammatory, immunological, antivenomous, antithrombotic, anticancer and antimalaria properties (Hosamani *et al*., 2011; Anurag *et al*., 2017; Sheeja *et al*., 2006; Kumar *et al*., 2001; Mishra *et al*., 2004). In recent times, the main alkaloid in *A. paniculata*; andrographolide has been reportedly confirmed for its anti-HIV activity (Nirlep, 2016).

Although many pharmacological activities of various solvent extracts of *Andrographis paniculata* have been reported, no literature has reported the antioxidant and antimicrobial activities of the various solvent fractions of the crude methanol extract of its leaves; hence the need for the present study.

2. Materials and Methods

2.1. Collection, Preparation and Extraction of Sample

Fresh and healthy leaves of *Andrographis paniculata* were obtained from its plant in a local farm in Ibadan, Oyo state, Nigeria and identified by a specialist in the Botany department of the University of Ibadan, Oyo state, Nigeria. The leaves were oven dried at 50 ^{o}C for 24 hours. 88 grams of the oven dried leaves was pulverized with the use of a laboratory blender (LEXUS MG-2053 OPTIMA) and extracted by maceration in 450 mL methanol, shaken and left for 48 hours. The mixture was filtered and the residue was re-macerated in another 350 mL methanol for 24 hours (three more times) in order to obtain adequate quantity of extract. The filtrates were combined and concentrated under reduced pressure at about 40 ^{o}C with the use of a vacuum rotary evaporator (Eyela N-1001) and this yielded a dark green semi-solid extract. The total average weight of the methanol extract obtained was 18.568 g.

2.2. Fractionation of Crude Methanol Extract

A portion of the crude methanol extract of *A. paniculata* leaves was reconstituted in distilled water and then fractionated successively (by liquid-liquid extraction method) into n-hexane, chloroform and ethyl acetate. Each of the resulting solvent fractions; n-hexane, chloroform, ethyl acetate as well as the water soluble fraction was collected and concentrated under reduced pressure at about 40 ^{o}C with the use of a vacuum rotary evaporator (Eyela N-1001). The methanol extract of *A. paniculata* and its fractions were immediately assayed for their antimicrobial and antioxidant activities using various standard methods.

2.3. Media Preparation

Nutrient agar and Potato Dextrose agar (Rapid Labs) were prepared following manufactures instruction. The media were sterilized in the autoclave at 121 ^{o}C for 15 minutes.

2.4. Antimicrobial Activity

Clinical isolates from stock cultures from Babcock University Teaching Hospital, Ilishan-Remo, Ogun State, Nigeria were used. These included *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter cloacae*, *Salmonella typhi* and *Candida albicans*. The already prepared agar plates were inoculated with 24 hour- old culture by uniformly streaking the surface of the agar in order to achieve uniform distribution of the test organism. A heat sterilized 10 mm cork borer was then used to make wells in the already inoculated medium with the number of wells bored and labelled corresponding to the number of concentrations of plant samples to be tested against each test organism. 100 µL of each concentration of the plant samples was then dispensed into corresponding wells of each set of organisms and allowed to stand for 30 minutes before being incubated at 37 ^{o}C for 24 hours. The inhibitory effect of the crude methanol extract of *A. paniculata* and its fractions on organism growth was assessed after 24 hours of incubation by visual analysis of the growth in each well and values were recorded. Dimethyl sulfoxide (DMSO) was used as the blank while 0.05 % ciprofloxacin was used as the positive control for bacteria while 0.05 % fluconazole for the fungus. All analyses were performed in triplicate. Minimum Inhibitory Concentration (MIC) of the extracts on the test organisms was done at varying concentrations and the results were recorded. The work benches were disinfected while the pathogenic organisms and the materials were autoclaved after use to avoid any form of contamination. Gloves and laboratory coats were also won as personal protective measures against the pathogenic organisms.

2.5. Antioxidant Activity

2.5.1. Free Radical Scavenging Activity

The ability of the crude methanol extract of *A. paniculata* and its fractions to scavenge free radicals was determined according to the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) spectrophotometric method of Mensor *et al*. (2001). One mL of a 0.3 mM DPPH methanol

solution was added to a 2.5 mL solution of the plant sample of various concentrations (200, 400, 600, 800 and 1000 µg/mL) or standard (2, 4, 6, 8 and 10 µg/mL), shaken thoroughly for one minute and allowed to react in the dark at room temperature for 30 minutes. The absorbance of the resulting mixture was measured at 518 nm on a UV-Visible spectrophotometer (JENWAY 6305) and converted to percentage antioxidant activity (AA %), using the formula:

$$AA\ \% = \frac{[(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100$$

Methanol (1.0 mL) plus extract solution (2.5 mL) was used as blank. 1ml of 0.3 mM DPPH plus methanol (2.5 mL) was used as a negative control. Standard solutions of Gallic acid served as positive controls. This assay was carried out in triplicates for each sample and concentration. The IC_{50} value represented the concentration of the plant sample (extract/ fraction) which scavenged 50 % of the DPPH free radical and this was obtained from the linear regression analysis (Stoilova *et al*., 2007).

2.5.2. Total Phenolic Content

The concentration of phenolics in plant sample (extract/fraction) of *A. paniculata* was determined using the method of Singleton *et al*. (1999). The reaction mixture was prepared by mixing 0.5 mL of methanol solution of extract/fraction (containing 100 µg/mL), 2.5 mL of 10 % Folin-Ciocalteu's reagent dissolved in water and 2.5 mL 7.5 % $NaHCO_3$. Blank containing 0.5 mL methanol, 2.5 mL 10 % Folin-Ciocalteu's reagent dissolved in water and 2.5 mL of 7.5 % of $NaHCO_3$ was concomitantly prepared. The samples were thereafter incubated in a thermostat at 45 ºC for 45 minutes. The absorbance was determined using spectrophotometer (JENWAY 6305) at a wavelength of 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solutions of Gallic acid to obtain a calibration curve (R^2 = 0.8752). Based on the measured absorbance, the concentration of phenolics was read from the calibration curve and expressed in terms of Gallic acid equivalent (mg of GA/g of extract/fraction).

2.5.3. Total Flavonoid Content

Analysis of total flavonoid content of the plant sample (extract/fraction) was done by using aluminum trichloride spectrophotometric method of (Dewanto *et al*., 2002). Quercetin was used as the reference substance. One milliliter of each sample in methanol (containing 100 µg/mL) was diluted with distilled water (4 mL) in a 10 mL volumetric flask. 5 % $NaNO_2$ solution (0.3 mL) was then added to each flask. At 5 minutes, 10 % $AlCl_3$ (0.3 mL) was added and at 6 minutes, 1.0 M NaOH (2 mL) was added. Distilled water (2.4 mL) was then added to the reaction flask and shaken thoroughly. Absorbance of the resulting reaction mixture was then read on a UV-Visible spectrophotometer (JENWAY 6305) at 510 nm. Reagent blank; containing 1 mL methanol in place of the extract was simultaneously prepared and treated in the same manner as the samples. A calibration curve was also prepared by repeating the same procedure for standard solutions of Quercetin (2 to 10 µg/mL, R^2 = 0.986). Based on the measured absorbance of the sample, the total Flavonoid Content was determined from Quercetin calibration curve and results expressed as mg Quercetin Equivalent per gram (mg QE g^{-1}) of the sample on a dry weight basis. The analysis was carried out in triplicates for each sample.

3. Results

The results of the percentage yield of methanol extract of *A. paniculata* as well as its various solvent fractions are shown in Table 1. The results showed that the yield of the methanol extract was 21.10 % of the extracted leaves while its solvent fractions; hexane, ethyl acetate, chloroform and water soluble were 13.10 %, 37.72 %, 11.87 % and 34.16 %, respectively.

Table 1. Percentage yield of methanol extract of *Andrographis paniculata* leaf and its fractions

Test sample	Percentage yield (%)
MEE	21.10±1.03
NHF	13.10±0.99
CHF	11.87±0.07
EAF	37.72±0.76
WSF	34.16±1.21

Data are expressed as mean ± standard error of three replicates. MEE= Crude methanol extract, NHF= n-Hexane fraction, CHF= Chloroform fraction, EAF= Ethyl acetate fraction, WSF= Water soluble fraction

Table 2 shows the results of the zones of inhibition of methanol extract of *A. paniculata* leaf and its fractions. *E. coli* showed resistance to all the test samples at the respective concentrations used. *Enterobacter cloacae* also showed resistance to the n-hexane fraction but showed susceptibility to methanol extract and the remaining fractions.

Table 3 shows the antimicrobial effect of 0.05 % Ciprofloxacin and 0.05% fluconazole on the test organisms. The standard antibiotic, Ciprofloxacin, was found to inhibit the growth of all the test bacteria except *Salmonella typhi* which showed resistance to the antibiotic while *Candida albicans* showed significant susceptibility to 0.05 % fluconazole.

The results presented in Table 4 shows the zones of inhibition of *Candida albicans* by methanol extract of *A. paniculata* leaf and its fractions. *Candida albicans* was resistant to methanol extract, n-hexane and chloroform fractions but was susceptible to ethyl acetate and water soluble fractions. The best inhibition of *Candida albicans* was, however, shown by the ethyl acetate fraction.

The results presented in Table 5 shows the MIC of the methanol extract and its fractions against the bacteria and *Candida albicans*. All the extracts showed MIC of 1 mg/mL for *Staphylococcus aureus* except chloroform which was 5 mg/mL. For *Salmonella typhi*, the MICs of methanol extract and chloroform fraction were 2 mg/mL and 4 mg/mL, respectively, while the MICs of other fractions was 1 mg/mL. All the extracts showed MIC of 1 mg/mL for *Enterobacter cloacae* except n-hexane and water soluble fractions with MIC values of > 20 and 5 mg/mL, respectively. On *Escherichia coli* all the samples

showed MIC of > 20 mg/mL. For *Candida albicans*, MIC values of ethyl acetate and water soluble fractions were 5 and 10 mg/ml, respectively. Meanwhile, methanol extract, n-hexane fraction and chloroform fraction did not show any inhibitions at concentrations lower than 20 mg/ml against *Candida albicans*.

The results of the total flavonoid and phenolic contents of the crude methanol extract of *A. paniculata* leaves and its fractions are represented in Table 6. The crude methanol extract showed the highest flavonoid content (41.79 µg QE/mg) while the least was shown by the chloroform fraction (11.66 µg QE/mg). The flavonoid contents of the others are: 28.77 µg QE/mg, 19.42 µg QE/mg and 17.11 µg QE/mg for water soluble fraction, ethylacetate fraction and n-hexane fraction respectively. However, n-hexane fraction showed the highest phenolic content (26.79 µg QE/mg) while chloroform fraction showed the least (16.17 µg GAE/mg). The crude methanol extract, ethylacetate fraction and water soluble fraction showed phenolic content of 24.96 µg GAE/mg, 20.54 µg GAE/mg, and 20.50 µg GAE/mg, respectively.

Meanwhile, the DPPH scavenging activity of the extract and its fraction are presented in Table 7. From the results, the crude methanol extract gave the best activity with an average percentage inhibition of 54.50 of the DPPH free radical. The ethyl acetate and water soluble fraction showed about the same DPPH scavenging activity while the least was shown by the n-hexane fraction with an average percentage inhibition of 11.36 of the DPPH free radical.

Table 2. Bacterial susceptibility pattern to methanol extract of *Andrographis paniculata* leaf and its fractions

Organism	Fraction	Diameter zones of inhibition (mm) at various concentrations of extract/ fractions			
		5 mg/mL	10 mg/mL	15 mg/mL	20 mg/mL
S. aureus	MEE	16±0.02	14±0.03	15±0.02	16±0.05
	NHF	13±0.12	14±0.07	14±0.01	14±0.11
	CHF	12±0.11	15±0.10	16±0.22	17±0.03
	EAF	14±0.02	15±0.02	15±0.13	15±0.11
	WSF	13±0.04	13±0.13	14±0.01	16±0.03
S. typhi	MEE	13±0.14	15±0.11	17±0.02	19±0.13
	NHF	11±0.02	15±0.02	15±0.04	18±0.04
	CHF	14±0.01	17±0.10	20±0.14	22±0.06
	EAF	17±0.22	19±0.04	22±0.21	23±0.05
	WSF	14±0.04	15±0.03	15±0.07	15±0.01
E. cloacae	MEE	15±0.01	16±0.01	17±0.01	17±0.05
	NHF	R	R	R	R
	CHF	15±0.12	16±0.04	16±0.05	17±0.01
	EAF	15±0.02	16±0.01	17±0.03	17±0.03
	WSF	12±0.02	13±0.01	13±0.04	13±0.01
E. coli	MEE	R	R	R	R
	NHF	R	R	R	R
	CHF	R	R	R	R
	EAF	R	R	R	R
	WSF	R	R	R	R

Data are expressed as mean ± standard error of three replicates. MEE= Crude methanol extract, NHF= n-Hexane fraction, CHF= Chloroform fraction, EAF= Ethyl acetate fraction, WSF= Water soluble fraction, R= Resistant

Table 3. Antimicrobial activities of 0.05 % Ciprofloxacin and 0.05 % fluconazole against the test organisms

Test organisms	Diameter zone of inhibition (mm)
Escherichia coli	22±1.43
Salmonella typhi	R
Staphylococcus aureus	19±1.22
Enterobacter cloacae	20±1.12
Candida albicans	21±0.55

Data are expressed as mean ± standard error of three replicates. MEE= Crude methanol extract, NHF= n-Hexane fraction, CHF= Chloroform fraction, EAF= Ethyl acetate fraction, WSF= Water soluble fraction, R= Resistant

Table 4. Susceptibility pattern of *Candida albicans* to the methanol extract of *Andrographis paniculata* leaf and its fractions

	Diameter zones of inhibition (mm) at various concentrations of extract/fractions (mg/ml)				
Test sample	20 mg/mL	40 mg/mL	60 mg/mL	80 mg/mL	100 mg/mL
MEE	R	R	R	R	R
NHF	R	R	R	R	R
CHF	R	R	R	R	R
EAF	11.5±0.51	12.5±0.51	13±0.13	14±0.66	17.5±0.81
WSF	11.5±0.81	12±0.11	12.1±0.21	12.2±0.022	13.0±0.02

Data are expressed as mean ± standard error of three replicates. MEE= Crude methanol extract, NHF= n-Hexane fraction, CHF= Chloroform fraction, EAF= Ethyl acetate fraction, WSF= Water soluble fraction, R= Resistant

Table 5. Minimum Inhibitory Concentration (mg/ml) of the methanol extract of *Andrographis paniculata* leaf and its fractions

Extracts	*Staphylococcus aureus*	*Salmonella typhi*	*Enterobacter cloacae*	*Escherichia coli*	*Candida albicans*
MEE	1	2	1	> 20	> 100
NHF	1	1	>20	> 20	> 100
CHF	5	1	1	> 20	> 100
EAF	1	1	1	> 20	5
WSF	1	4	5	> 20	10

MEE= Crude methanol extract, NHF= n-Hexane fraction, CHF= Chloroform fraction, EAF= Ethyl acetate fraction, WSF= Water soluble fraction

Table 6. Phenolic and Flavonoid contents of methanol extract of *Andrographis paniculata* leaf and its fractions

Extracts (1000 µg/ml)	Flavonoid (µg QE/mg)	Phenolic (µg GAE/mg)
Methanol extract	41.79±0.44	24.96±1.00
Hexane fraction	17.11±0.51	26.79±0.66
Ethyl acetate fraction	19.42±0.21	20.54±0.51
Chloroform fraction	11.66±0.12	16.17±0.61
Residual fraction	28.77±0.35	20.50±0.43

Data are expressed as mean ± standard error of three replicates. MEE= Crude methanol extract, NHF= n-Hexane fraction, CHF= Chloroform fraction, EAF= Ethyl acetate fraction, WSF= Water soluble fraction

Table 7. DPPH Scavenging activity of methanol extract of *Andrographis paniculata* leaf and its fractions

Extract	Average % inhibition of DPPH	IC50 (µg/mg)
Methanol	54.50±0.10	536.04±1.11
N-Hexane	11.36±0.10	4422.38±1.24
Ethyl acetate	42.61±0.21	703.34±1.44
Chloroform	26.97±0.20	1166.17±1.34
Residual	42.60±0.71	1203.24±1.11

Data are expressed as mean ± standard error of three replicates. MEE= Crude methanol extract, NHF= n-Hexane fraction, CHF= Chloroform fraction, EAF= Ethyl acetate fraction, WSF= Water soluble fraction

4. Discussion

The emergence of multi-drug resistance in human pathogens which threatens the efficacy of commonly used antibiotics (Bandow *et al*., 2003) and the increasing cost of synthetic drugs have necessitated a search for new antimicrobial substances from other sources, especially plants, by pharmaceutical industries. Plants are known to possess a variety of compounds to protect themselves against a variety of their own pathogens and can therefore be considered as potential sources of different classes of pharmaceutical substances. Many synthetic drugs are usually accompanied with a number of side effects when compared with medicinal plants which on the other hand are natural and are perceived to have little or no side effects; they are considered safer, easily accessible and of lower cost (Ghosh *et al*., 2008; Kumar and Pandey, 2012). However, others have argued that determining the precise pharmacological activity and side effects or toxicity of a singular active chemical compound usually present in synthetic drugs is considerably easier as against numerous chemicals normally contained in medicinal plants (Philomena, 2011). This may be attributed to the complexity of interactions and synergies that might occur amongst the numerous chemicals found in crude plant extracts (Philomena, 2011). Several studies have reported that medicinal plants have one or more of their parts which

contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowora, 2008). However, the selective extraction or isolation of each individual pharmaceutical compound from medicinal plants could be time consuming, stressful and capital intensive.

In the present study, leaves of *A. paniculata* were extracted with methanol. The crude methanol extract of the leaves was then reconstituted in water and successively partitioned with n-hexane, chloroform and ethyl acetate to give n-hexane, chloroform, ethyl acetate and water soluble fractions. The crude methanol extract and its fractions were analyzed for their antimicrobial and antioxidant activities. The results of the present study revealed that the extract of *A. paniculata* leaf and its resulting fractions had a significant antimicrobial activity on the test organisms as well as considerably promising antioxidant potential. This could be due to the presence of significant amount of polyphenolic compounds determined in the extract and fractions of *A. paniculata*. Polyphenolic compounds have been reported to serve many functions in plants; some of which are cell wall strengthening, antibacterial and antifungal activities (Furiga *et al*., 2008). Due to the complexity of compound mixture in the plant extract, antioxidant and antimicrobial potential of its fractions did not follow a particular order. This may also be due to the difference in the polarity of the partitioning solvent which may result in extraction of compounds of varied properties in the different fractions.

Andrographis paniculata has been reported to have antibacterial effect on both Gram positive and Gram negative bacteria (Aniel *et al*., 2010; Neha, 2016). In the present study, all the test organisms were susceptible to the test samples except *E. coli* which showed complete resistance to the crude methanol extract and its entire fractions at the studied concentrations. This is in consonant with the findings of (Suparna *et al*., 2014) who also reported strong resistance of *E. coli* to leaf extracts of *A. paniculata*. *Salmonella typhi* on the other hand showed the highest susceptibility to the crude extract as well as its fractions especially at 20 mg/mL concentration. Other studies, carried out on the leaf of *A. paniculata*, used higher concentrations: 100, 200 and 500 mg/mL (Suparna *et al*., 2014) and 750 mg/mL (Aniel *et al*., 2010). It is generally known that the activities of antimicrobials increase with concentration. The results of the present study, however, showed that the inhibitory activity of the crude extract and its fractions against the test organisms was slightly concentration dependent. It is important to note also that *C. albicans* showed strong resistance to all the test samples except the ethyl acetate and water soluble fractions. Ethyl acetate fraction, however, showed the best anti-*Candida* activity. This may imply that ethyl acetate was the most suitable solvent for the extraction of compounds with good anti- *Candida* activities from the crude methanol extract of *A. paniculata*. *Salmonella typhi* showed resistance to the standard substance; 0.5 % Ciprofloxacin, but was susceptible to all the test samples. This might be due to the large array of compounds in the complex mixture of the fractions which may work synergistically to enhance the antimicrobial potency of the fractions. Meanwhile, the importance of efficient liquid-liquid separations has been pointed out to be critical in achieving optimum plant performance (Cusack *et al*., 2009) especially for pharmacological purposes.

The relatively high yield obtained for crude methanol extract in the present study may be due to the ability of methanol to extract both polar and non-polar compounds from plants. Previous authors (Siddhuraju and Becker, 2003) reported the efficiency of methanol for extracting high amount of pharmaceutically important phtyochemicals, such as the polyphenolic compounds from plants. However, the highest and lowest fraction yields were obtained for ethyl acetate and chloroform fractions of the crude methanol extract, respectively. This variation in yield may also be due to variations in polarity of the partitioning solvents used as well as differences in extractability of bioactive compounds. The polarity of extraction solvents has been suggested to play an important role in the ability of plant extracts to exhibit potential antimicrobial activities (Siddhuraju and Becker, 2003; Jigna *et al*., 2006; Sultana *et al*., 2007).

Antioxidants protect the body from the damaging effect of free radicals either by suppressing the formation of the free radicals, scavenge them before they do damage to body cells or repair damage that has been done by them. Medicinal plants are known to contain loads of phytochemicals with outstanding antioxidant properties. One of the most important groups of these phytochemicals is the polyphenolics which are renowned for their free radical scavenging ability (Ravipati *et al*., 2012; Ogasawara *et al*., 2007). In the present study, the free radical scavenging ability of the crude methanol extract and fractions was determined through the degree of discoloration of the methanol solution of DPPH. In the presence of an active free radical scavenger, the absorption vanishes and the resulting discoloration is stoichiometric at a selected range with respect to the degree of reduction (Janaina *et al*., 2009). The solution loses color with increase in concentration of antioxidant as electrons are taken up by DPPH radical from the antioxidant (Calliste *et al*., 2001). The present study reveals that the best antioxidant activity in terms of DPPH scavenging strength was displayed by methanol extract. This could be attributed to its possession of the highest flavonoid content. Flavonoids are a group of polyphenols with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory actions (Wang *et al*., 2006; Frautchy *et al*., 2001; Clavin *et al*., 2007). However, even though the n-hexane fraction contained the highest phenolic content, it showed the least free radical scavenging strength. It is therefore important to note that flavonoid and phenolic are not the only phytochemicals that confer antioxidant properties on plants. Other classes of phytochemicals, such as carotenoids, tannins, volatile oils, α-tocopherols, and ascorbic acid have also been reported to enhance the antioxidant ability of plants (Javanmardi *et al*., 2003; Amarowicz, 2007). Recent findings suggest that diets rich in polyphenolic substances play an important role in combating oxidative stress related disorders due of their antioxidant activities. Hence, polyphenolic constituents of *A. paniculata* could possess the capability to counteract oxidative stress related disorders.

5. Conclusion

The crude methanol extract of *A. paniculata* leaf and its fractions showed considerable antimicrobial and antioxidants activities. The Ethyl acetate fraction in comparison with other fractions showed the best antimicrobial activities. It was therefore concluded that the ethyl acetate fraction of the leaves' crude methanol extract contained most of the bioactive components with both antibacterial and anti-Candida activities. However, the best antioxidant activity was exhibited by the crude methanol extract and this was attributed to its possession of highest flavonoid content. The present study further supports the traditional use of this plant for the treatment of various infections and diseases, such as food poisoning, typhoid, diarrhea, urinary tract infection, boil, skin rashes, inflammation, aging, heart disease, cataracts etc. for which the test organisms (bacteria and fungus) and free radicals may be implicated or associated with. The present study of antioxidant and antimicrobial evaluation of the different fractions of methanol extract of *A. paniculata* leaves forms a primary platform for further phytochemical and pharmacological studies. In addition to carrying out researches on the phytotoxicity of the plant as well as establishing a safe dosage regime, further works on the characterization, isolation and purification of the active compounds from the extract is imperative. This would pave way for further evidence based investigations to ascertain whether whole plant extracts are better for pharmacological purposes than pure compounds extracted from them or vice versa.

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