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Comparisons Between Different Crude Extract Yields Of Whole Plant Of Momordica Charantia And Their Antibacterial Susceptibility Against Selected Clinical Isolates

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Abstract
Medicinal plants such as *Momordica charantia* contained different phytochemicals which have antibacterial activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas fluorescens, Salmonella typhimurium* and *Klebsiella pneumoniae*. In this study, four solvents namely *n*-hexane, ethyl acetate, methanol and aqueous were used for the reflux extraction of whole plant of *M. charantia* successively and exhaustively. The phytochemicals and antibacterial susceptibility of the crude extracts against selected clinical isolates were determined at different concentrations of 40 mg/ml, 60 mg/ml, 80 mg/ml and 100 mg/ml using agar well diffusion. The clinical isolates used were subjected to biochemical tests and molecular characterization for proper identification. The study revealed that ethyl acetate extract had the highest percentage yield of 5.92% followed by 3.33% of aqueous extract. Phytochemicals such saponins, flavonoids, terpenoids, cardiac glycosides, tannins, phenols, alkaloids, steroids and reducing sugars were present in the crude extracts. The highest mean and standard error of means value of antibacterial susceptibility against *E. coli* were 14.67±0.33; 19.67±0.33; 21.67±0.33 and 30.33±0.33 for 40 mg/ml, 60 mg/ml, 80 mg/ml and 100 mg/ml concentrations of the aqueous crude extract respectively. This research work showed that there are differences in the yields of crude extracts of whole plant of *M. charantia* when extracted with different solvents and the antibacterial activity result showed that the aqueous crude extract has a better antibacterial activity against the selected clinical isolates than other crude extracts. This present study compared the different yields of *M. charantia* and its antibacterial susceptibility against selected clinical isolates.

Keywords:
Comparisons, solvents, antibacterial susceptibility, *Momordica charantia*, clinical isolates

INTRODUCTION

Altundağ and Öztürk (2011) reported that from time immemorial; plants and plant based products are used in the treatment and prevention of illness. The secondary metabolites in plants are the cornerstone of many treatment modalities like Homeopathy, Allopathy, Unani- Ayurvedic medicine. The developed and developing countries are faced with microbial infections that constitute major public health problems (Altundağ and Öztürk, 2011). As a result of the general use of commercial antibiotics, the relative incidence of multiple antibiotic resistances in human pathogens is not only large but keeps growing as reported by Li and Krumholz (2007). The intractable problems of resistance to antibiotics by microorganisms previously thought to have been overcome have led to the resurface of interest in herbal products as source of potential compounds to suppress or possibly eradicate the ever increasing problems of emergence of newer diseases as reported by Oyama *et al*. (2016). According to World Health Organization (WHO) (2003), about 80% of people still rely mainly on traditional remedies such as herbs for their medicines. Al-Sayed *et al*. (2014) opined that most of the plants used by the rural residential areas have biologically active compounds that have been proven by generations to be potent against specific disorders. In fact, the prevention and treatment of diseases by the use of available and accessible medicinal plants in a particular locality will continue to play important roles in medical health care implementation in the developing countries as plants make up the primary source of new pharmaceuticals and health care products (Yankova, 2005). Helal *et al*. (2015) reported that natural products are therefore gaining attention as an alternative for antimicrobial agents.

M. charantia commonly known as bitter melon, bitter gourd or balsam pear is a medicinal plant belonging to the family *Cucurbitaceae*. In Nigeria it is called by its local names which include "Ejirin" (Yoruba), "Daladdasu" (Hausa), "Garafinni" (Nupe) and "Okwunuolo" (Igbo). It is indigenous to tropical and subtropical regions of the world such as India, Asia, South America and West Africa and widely used as food and medicine (Adegbola *et al*., 2016).

M. charantia extracts and juice has been found suitable for different diseases and problems. Several researchers had reported the effectiveness of its extracts in the treatment of ailments such as lowering of blood sugar or other actions of potential benefit against diabetes mellitus due to its hypoglycemic properties (Leatherdale *et al.,* 2001; Bachok *et al.,* 2014); controlling eye disorders and enhancing eyesight due to the presence of beta-carotene (Leatherdale *et al.,* 2001); diarrhea, pyorrhea that is bleeding from the gums (Welhinda *et al.,* 2002); piles and hemorrhoids (Srivastava *et al*., 1996); respiratory problems (Jayasooriya *et al*., 2000) and skin infections (Ahmad *et al*., 1999). Zhu *et al*. (1990) had also reported the anti-cancerous and anti-leukemic activity of bitter melon against numerous cell lines including liver cancer, human leukemia melanoma and solid sarcomas. It has been seen that it has strong antimicrobial activity against wide range of gram positive and gram negative bacteria (Taylor, 2000). It contained antibiotic and anti-tumor activities. In addition to these properties, leaf extracts of bitter melon have clinically demonstrated broad spectrum antimicrobial activity. Various extracts of water, ethanol and methanol of the leaves have demonstrated in vitro antibacterial activities against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium, Pseudomonas fluorescens, Klebsiella pneumoniae and Bacillus subtilis,* an extract of the entire plant was shown to have antiprotozal activity against *Entamoeba histolytica*. In other study, the fruit extract has demonstrated activity against the stomach ulcer-causing bacteria *Helicobacter pylori* (Yesilada *et al.,* 1999).

According to Mamta *et al*. (2013), phytochemicals which are plant-derived substances have recently become great interest owing to their versatile application. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids and chlorophyll's (Mamta *et al*., 2013). Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids and glucosides. Literature survey indicate that phenolics are the most numerous and structurally diverse plant phytoconstituents (Mamta *et al*., 2013).

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Fresh whole plant *M. charantia* plants were collected in Bosso within Minna Metropolis. The plant materials were taken to the Department of Biological Sciences in Federal University of Technology, Minna for botanical identification. The identified plants were authenticated by the Herbarium Department of National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria with the voucher number NIPRD/H/6862 and the voucher specimen deposited in the same Herbarium Department. The identified/authenticated medicinal plant was thoroughly washed with water to remove any extraneous substances and the whole plants dried at room temperature away from sunlight and dust for sixteen weeks until a constant weight was obtained. .

Preparation of Extracts

The air dried whole plants were grounded to powder using a blender. The extraction was done using reflux extraction method as described in Ewansiha *et al*. (2016). The reflux extraction method was used to obtain the crude extract at a reduced temperature for 2 hours. The extraction was carried out beginning from non-polar solvent to polar solvent. The solvents used were *n*-hexane, ethyl acetate, methanol and water. One hundred grams (100 g) of the well blended dried plant material was dissolved in 400 ml of the extracting solvent (for example, *n*-hexane, ethyl acetate, methanol and water). At the end of 2 hours of refluxing, the mixture was filtered firstly with muslin cloth and later with Whatman No. 1 filter paper with pore size 0.7 µm in order to obtain a clear filtrate which was then concentrated to a semi-solid substance with the use of a Rotary Evaporator (model RE-6000) at a reduced temperature and then dried using water bath set at 60^{o}C. The dried extract was then stored in an air-tight amber bottle and kept in the refrigerator for further analysis. The marc was air-dried for 1 hour and was extracted with the next solvent in increasing polarity. This procedure was repeated using other solvents.

Determination of the Percentage Yield of Crude Extracts

The percentage yield of crude extracts was determined using the equation: Percentage Yield = [(Weight of extract/Weight of dry plant material) x 100] (Ewansiha *et al*., 2016).

Determination of the Phytochemical Components in the Crude Extracts

The crude extracts were subjected to phytochemical analysis to screen for the presence of secondary metabolites such as alkaloids, saponins, phenols, reducing sugars, flavonoids, cardiac glycosides, terpenoids, steroids and tannins. The phytochemical screening was carried out using standard procedure as reported by Evans (2002).

Collection, Gram Staining, Biochemical Tests, Molecular Characterization and Identification of Selected Clinical Isolates

The *Escherichia coli*, *Pseudomonas fluorescens*, *Klebsiella pneumoniae*, *Salmonella typhimurium* used were collected from the Microbiology Department of General Hospital, Minna. They were Gram stained and their biochemical tests were carried out in order identify them according to the methods of Cheesbrough (2010) and then sub cultured into Nutrient Agar slants for molecular characterization for confirmation (Bioneer Incorporation, 2012; Beckman Coulter Incorporation, 2016; National Centre for Biotechnology Information, 1988).

Standardization of Selected Clinical Isolates

The population of the clinical isolates were determined from the 0.5 McFarland Turbidity Standard prepared (Murray *et al*., 2007). The clinical isolates were cultured for 24 h in a Nutrient Broth and then ten-fold dilutions from 10^{-1} to 10^{-6} were made. The absorbance of each of the dilution was determined at 540 nm using Jenway 6305 UV/Visible Spectrophotometer and then compared with the absorbance of the 0.5 McFarland Turbidity Standard prepared.

Media Preparation

Mueller Hinton Agar (MHA) and Nutrient Broth were prepared and sterilized according to the manufacturer's instructions.

Preparation of Extract Concentration

The extract concentration was prepared as described in the work of Ewansiha *et al*. (2016). Two hundred milligram (200 mg) of the n-hexane, ethyl acetate, methanol and aqueous crude extract were weighed in 5 ml each of 20% Dimethyl sulfoxide (DMSO) (20 ml DMSO was made up to 100 ml with distilled water) to give 40 mg/ml concentrations respectively. Similar preparations were made for 60 mg/ml, 80 mg/ml and 100 mg/ml concentrations.

Determination of the Antibacterial Susceptibility of the Crude Extracts

The antibacterial susceptibility of the crude extract was carried out using Agar Well Diffusion method outlined in CLSI (2015). MHA was prepared and sterilized as instructed by the manufacturer. Petri dishes containing about 20 ml MHA were streaked with standardized 24 h culture of the clinical isolates using sterile swab sticks. Wells were cut with a 6 mm sterile cork borer and then sealed at the bottom with a drop of molten agar so as to prevent the extract from sipping beneath the agar. Four holes were made on each plate and adequately spaced out. About 100 µl of the crude extracts (40, 60, 80 and 100 mg/ml of aqueous, methanol, n-hexane and ethyl acetate extracts) was introduced into each well; 100 µl of 40, 60, 80 and 100 mg/ml of the standard drug (Ciprofloxacin) were used for positive controls and dimethylsulfuroxide (DMSO) served as the negative control. One hour pre-diffusion time was allowed after which the plates were incubated at 37°C for 24 h. The zones of inhibition were measured by direct linear measurement using a meter scale rule. The above method was carried out in triplicates and the mean of the triplicate result were taken (CLSI, 2015).

RESULTS

Percentage Yield of Crude Extracts

The percentage yield of different extracts is shown in Table 1. The Normal Hexane Extract had 0.66%; Ethyl Acetate Extract recorded 5.92%; Methanol Extract and Aqueous Extract had 2.30% and 3.33% respectively (Table 1).

Table 1: Percentage Yield of Crude Extracts

Crude Extracts	Yield (g)	Percentage Yield (%)
n-Hexane Extract	0.6605	0.66
Ethyl Acetate Extract	5.9193	5.92
Methanol Extract	2.3008	2.30
Aqueous Extract	3.3299	3.33

Phytochemical Components in *M. charantia* Whole Plant Crude Extracts

Table 2 showed the phytochemical components found in *M. charantia* whole plant crude extracts. The *n*-hexane crude extract (NHE) had terpenoids, steroids, tannins, cardiac glycosides and flavonoids whereas phenols, saponins, alkaloids and reducing sugars were absent. On the other hand, alkaloids, flavonoids, phenols, cardiac glycosides, terpenoids, saponins and steroids were present in ethyl acetate crude extract (EAE) but reducing sugars and tannins were absent. Table 2 showed that methanol crude extract (ME) had saponins, flavonoids, terpenoids, tannins, phenols, alkaloids, steroids and cardiac glycosides except reducing sugars that were absent. The aqueous crude extract (AE) contained saponins, flavonoids, terpenoids, tannins, phenols, alkaloids, cardiac glycosides, reducing sugars and steroids (Table 2).

Table 2: Phytochemicals in *M. charantia* whole plant extracts using different Solvents

Extracts	Phytochemicals								
	Saponins	Flavonoids	Terpenoids	Cardiac Glycosides	Tannins	Phenols	Alkaloids	Steroids	Reducing Sugars
***n*-hexane**	-	+	+	+	+	-	-	+	-
Ethyl acetate	+	+	+	+	-	+	+	+	-
Methanol	+	+	+	+	+	+	+	+	-
Aqueous	+	+	+	+	+	+	+	+	+

KEY: + Present, - Absent

3.3 Gram Staining and Biochemical Tests of the Clinical Isolates

The result of the Gram stain reactions and biochemical tests conducted on the four clinical isolates is shown in Table 3. The four clinical isolates were Gram negative. Six tests which include triple sugar iron (TSI); methyl red; indole; catalase; citrate utilisation and urease were carried out on the isolates for confirmation and identification. The 4 isolates tested for TSI were all positive. *E. coli* and *S. typhimurium* were positive for methyl red while *K. pneumoniae* and *P. fluorescens* were negative. Table 3 also revealed that *E. coli* was indole positive while *P. fluorescens, S. typhimurium* and *K. pneumoniae* were indole negative. However, all the isolates were catalase positive as revealed in Table 3. The citrate utilisation test was positive in *P. fluorescens* and *K. pneumoniae* but *E. coli* and *S. typhimurium* were negative (Table 3). The urease test was positive in *E. coli*, *P. fluorescens* and *K. pneumoniae* but negative in *S. typhimurium* (Table 3).

Table 3: Gram Reactions and Biochemical Tests Conducted on the Bacteria Isolates

Tests	*E. coli*	*P. fluorescens*	*S. typhimurium*	*K. pneumoniae*
Gram Reactions	-	-	-	-
TSI	+	+	+	+
Methyl Red	+	-	+	-
Indole	+	-	-	-
Catalase	+	+	+	+
Citrate Utilisation	-	+	-	+
Urease	+	+	-	+

KEY:
+ = Positive; - = Negative

Molecular Characterization of the Clinical Isolates

The result of clinical isolates characterized molecularly is shown in Table 4. The sequence BLAST results revealed the identity of the bacterial isolates with their accession numbers as follows: *E. coli* strain MRE 600 (CP014197.1); *P. fluorescens* strain 2P24 (CP025542.1); *Salmonella* enteric subsp. enteric serovar *typhi* PMO 16/13 (CP12091.1) and *K. pneumoniae* strain HZW25 (CP025211.1)

Table 4: Identity and Accession Numbers of Molecularly Characterized Clinical Isolates

Bacterial Isolates	Max Score	Total Score	Query Cover	Expected Value	Identity	Accession
E. coli strain MRE 600	2660	16064	100%	0.0	100%	CP014197.1
P. fluorescens strain 2P24	2689	2689	100%	0.0	100%	CP025542.1
Salmonella enteric subsp. enteric serovar *typhi* PMO 16/13	3546	3546	100%	0.0	100%	CP12091.1
K. pneumoniae strain HZW25	797	6348	100%	0.0	98%	CP025211.1

Antibacterial Activity of Whole Plant Crude Extracts of *M. charantia*

Tables 5-8 showed the results of antibacterial activity of *n*-hexane extract (NHE), ethyl acetate extract (EAE), methanol extract (ME) and aqueous extract (AQE) of whole plant crude extracts of *M. charantia* tested against standardized *S. typhimurium*, *E. coli*, *P. fluorescens* and *K. pneumoniae* using 40 mg/ml; 60 mg/ml; 80 mg/ml and 100 mg/ml.

Table 5 showed that there was no antibacterial activity with NHE, EAE and ME but 14.67±0.33 was recorded with AQE when tested against standardized *E. coli* when 40 mg/ml concentration was used. *S. typhimurium* had no antibacterial activity with NHE, EAE and ME but 13.67±0.33 was recorded with AQE (Table 5). *K. pneumoniae* and *P. fluorescens* had no antibacterial activity with the four crude extracts (Table 5). The DMSO (serving as negative control) showed no antibacterial activity while the 40 mg/ml of Ciprofloxacin (serving as positive control) showed antibacterial activity with the mean zones of inhibition and standard error of means of 11.67±0.33; 45.33±0.33; 50.00±0.00 and 34.67±0.33 for *E. coli*, *S. typhimurium*, *K. pneumoniae* and *P. fluorescens* respectively (Table 5).

Table 5: Mean Zones of Inhibition of Whole Plant Crude Extracts (40 mg/ml) of *M. charantia* (mm)

Extracts/Control	Test Organisms			
	E. coli	*S. typhimurium*	*K. pneumoniae*	*P. fluorescens*
***n*-Hexane**	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
Ethyl Acetate	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
Methanol	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
Aqueous	14.67±0.33^{a}	13.67±0.33^{b}	0.00±0.00^{b}	0.00±0.00^{b}
Ciprofloxacin	11.67±0.33^{b}	45.33±0.33^{a}	50.00±0.00^{a}	34.67±0.33^{a}
DMSO	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}

*Results represent Mean ± Standard Error of Mean of triplicate determination. Values with the same superscript in the same column are not significantly different at $p<0.05$

Table 6 showed that *E. coli* had no antibacterial activity with NHE but 9.67±0.33, 12.33±0.60 and 19.67±0.33 were recorded with EAE, ME and AQE respectively when 60 mg/ml concentration was used. *S. typhimurium* had no antibacterial activity with 60 mg/ml of NHE and EAE but had 11.67±0.33 and 15.33±0.33 with ME and AQE respectively (Table 6).

Table 6 further revealed that there was no antibacterial activity with NHE, EAE, ME and AQE when tested against *K. pneumoniae* and *P. fluorescens* respectively (Table 6). The DMSO (serving as negative control) showed no antibacterial activity while 60 mg/ml of Ciprofloxacin (serving as positive control) showed antibacterial activity with the mean zones of inhibition and standard error of means of 44.33±0.67; 46.00±0.33; 52.00±0.58 and 35.00±0.58 for *E. coli*, *S. typhimurium*, *K. pneumoniae* and *P. fluorescens* respectively (Table 6).

Table 6: Mean Zones of Inhibition of Whole Plant Crude Extracts (60 mg/ml) of *M. charantia* (mm)

Extracts/Control	Test Organisms			
	E. coli	*S. typhimurium*	*K. pneumoniae*	*P. fluorescens*
***n*-Hexane**	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{b}	0.00±0.00^{b}
Ethyl Acetate	9.67±0.33^{d}	0.00±0.00^{d}	0.00±0.00^{b}	0.00±0.00^{b}
Methanol	12.33±0.6^{c}	11.67±0.33^{c}	0.00±0.00^{b}	0.00±0.00^{b}
Aqueous	19.67±0.33^{b}	15.33±0.33^{b}	0.00±0.00^{b}	0.00±0.00^{b}
Ciprofloxacin	44.33±0.67^{a}	46.00±0.58^{a}	52.00±0.58^{a}	35.00±0.58^{a}
DMSO	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{b}	0.00±0.00^{b}

*Results represent Mean ± Standard Error of Mean of triplicate determination. Values with the same superscript in the same column are not significantly different at p<0.05

Tables 7 showed the antibacterial activity of 80 mg/ml of each of the crude extracts tested against standardized *E. coli*, *S. typhimurium*, *K. pneumoniae* and *P. fluorescens* respectively. *E. coli* showed antibacterial activity values of 11.33±0.33, 14.67±0.33, 14.67±0.33 and 21.67±0.33 with NHE, EAE, ME and AQE respectively (Table 7). *S. typhimurium* had no antibacterial activity with NHE but recorded 21.67±0.33, 13.00±0.00 and 16.33±0.33 with EAE, ME and AQE respectively (Table 7. However, *K. pneumoniae* and *P. fluorescens* had no antibacterial activity with the four crude extracts (Table 7). The DMSO (serving as negative control) showed no antibacterial activity while 80 mg/ml of Ciprofloxacin (serving as positive control) showed antibacterial activity with the mean zones of inhibition and standard error of means of 46.00±0.58; 48.00±0.58; 53.00±0.33 and 37.00±0.58 for *E. coli*, *S. typhimurium*, *K. pneumoniae* and *P. fluorescens* respectively (Table 7).

Table 7: Mean Zones of Inhibition of Whole Plant Crude Extracts (80 mg/ml) of *M. charantia* (mm)

Extracts/Control	Test Organisms			
	E. coli	*S. typhimurium*	*K. pneumoniae*	*P. fluorescens*
***n*-Hexane**	11.33±0.33^{d}	0.00±0.00^{e}	0.00±0.00^{b}	0.00±0.00^{b}
Ethyl Acetate	14.67±0.33^{c}	21.67±0.33^{c}	0.00±0.00^{b}	0.00±0.00^{b}
Methanol	14.67±0.33^{c}	13.00±0.00^{d}	0.00±0.00^{b}	0.00±0.00^{b}
Aqueous	21.67±0.33^{b}	16.33±0.33^{b}	0.00±0.00^{b}	0.00±0.00^{b}
Ciprofloxacin	46.00±0.58^{a}	48.00±0.58^{a}	53.00±0.33^{a}	37.00±0.58^{a}
DMSO	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{b}	0.00±0.00^{b}

*Results represent Mean ± Standard Error of Mean of triplicate determination. Values with the same superscript in the same column are not significantly different at p<0.05

Table 8 revealed the antibacterial activity of 80 mg/ml of each of the crude extracts tested against standardized *E. coli*, *S. typhimurium*, *K. pneumoniae* and *P. fluorescens* respectively. *E. coli* showed antibacterial activity values of 12.67±0.33, 18.33±0.88, 19.33±0.33 and 30.33±0.33 with NHE, EAE, ME and AQE respectively (Table 8). *S. typhimurium* had antibacterial activity values of 12.00±0.58, 23.67±0.33, 18.33±0.88 and 16.67±0.33 with NHE, EAE, ME and AQE respectively (Table 8). However, *K. pneumoniae* had no antibacterial activity with NHE and ME but recorded 17.33±0.67 and 14.00±0.58 with EAE and AQE (Table 8).

However, *P. fluorescens* had no antibacterial activity with the four crude extracts (Table 8).The DMSO (serving as negative control) showed no antibacterial activity while 100 mg/ml of Ciprofloxacin (serving as positive control) showed antibacterial activity with the mean zones of inhibition and standard error of means of 49.67±0.33; 48.67±0.33; 55.00±0.58 and 38.33±0.67 for *E. coli*, *S. typhimurium*, *K. pneumoniae* and *P. fluorescens* respectively (Table 8).

Table 8: Mean Zones of Inhibition of Whole Plant Crude Extracts (100 mg/ml) of *M. charantia* (mm)

Extracts/Control	Test Organisms			
	E. coli	*S. typhimurium*	*K. pneumoniae*	*P. fluorescens*
***n*-Hexane**	12.67±0.33^{e}	12.00±0.58^{e}	0.00±0.00^{d}	0.00±0.00^{b}
Ethyl Acetate	18.33±0.88^{d}	23.67±0.33^{b}	17.33±0.67^{b}	0.00±0.00^{b}
Methanol	19.33±0.33^{c}	18.33±0.88^{c}	0.00±0.00^{d}	0.00±0.00^{b}
Aqueous	30.33±0.33^{b}	16.67±0.33^{d}	14.00±0.58^{c}	0.00±0.00^{b}
Ciprofloxacin	49.67±0.33^{a}	48.67±0.33^{a}	55.00±0.58^{a}	38.33±0.67^{a}
DMSO	0.00±0.00^{f}	0.00±0.00^{f}	0.00±0.00^{d}	0.00±0.00^{b}

*Results represent Mean ± Standard Error of Mean of triplicate determination. Values with the same superscript in the same column are not significantly different at $p<0.05$

DISCUSSION

Table 1 showed that there were differences in the percentage yield of crude extracts of *M. charantia*. The differences between the yields of extracts might be attributed to the availability of different extractable components as opined by Oke and Aslim (2010). Table 1 showed that the percentage yield obtained from aqueous used as a solvent which was closely followed by the yield from ethyl acetate suggests that the aqueous is also a better solvent. The difference in solvents and methodology used in this study might possibly be responsible for the differences in the yield of crude extracts of *M. charantia*.

Table 2 showed that whole plant crude extracts of *M. charantia* contained saponins, flavonoids, terpenoids, cardiac glycosides, tannins, phenols, alkaloids, steroids and reducing sugars. This result is in agreement with the work of Mada *et al*. (2013) and Abalaka *et al*. (2017). However, the results differ from the work of Abalaka *et al*. (2012) in which the crude extracts had in addition to saponins phlobatannins. Although, the quantities of the phytochemicals vary in the four crude extracts yet all the extracts contained, flavonoids, cardiac glycosides, steroids and terpenoids as shown in Table 2. On the other hand, the results differ from that of Adegbola *et al*. (2016) in which cardiac glycosides were absent completely in the crude extracts. The presence of these phytochemicals is in line with work of Ewansiha *et al*. (2016). In the same vein, the presence of other bioactive agents in the whole plant extracts of *M. charantia* is in line with the work of Ukoha *et al*. (2011) and Abalaka *et al*. (2017).

Flavonoids are an integral phytochemical constituent of higher plants. They have antioxidant potentials hence could offer protection against heart disease and cancer probably by enhancing the body defence against pathology induced free radicals generation as opined by Al-Humaid *et al*. (2010). As reported by Mamta *et al*. (2013), the tannin-containing plant extracts are used as astringents, against diarrhoea, as diuretics, against stomach and duodenal tumours and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals. Phenols are famous group of secondary metabolites with wide pharmacological activities (Ghasemzadeh *et al*., 2010; Silva *et al*., 2007). In addition, alkaloids have many pharmacological activities including antihypertensive effects (many indole alkaloids), antiarrhythmic effect (quinidine, spareien), antimalarial activity (quinine) and anticancer actions (dimeric indoles, vincristine, vinblastine) as reported in the work of Mamta *et al*. (2013).

Furthermore, Quinlan *et al*. (2000) worked on steroidal extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates. Neumann *et al*. (2004) also confirmed the antiviral property of steroids. In this study both biochemical and molecular characterization confirmed the clinical isolates to be *E. coli*; *S. typhimurium*; *P. fluorescens* and *K. pneumonia*e (Table 3).

The use of biochemical and molecular approach in identifying bacteria is in conformity with the report by Ewansiha *et al*. (2016).

The results of antibacterial activity of whole plant crude extracts of *M. charantia* in Tables 5-8 showed that *n*-hexane extract (NHE), ethyl acetate extract (EAE), methanol extract (ME) and aqueous extract (AQE) had no antibacterial activity when tested against *P. fluorescens* despite varying the concentrations from 40 mg/ml to 100 mg/ml. In the same vein, NHE, EAE, ME and AQE had no antibacterial activity when tested against *K. pneumoniae* as shown in Tables 5-8. This result contradicts the work of Adegbola *et al*. (2016) and Mada *et al*. (2013). This might be due to the test organisms used and the types of solvents used for the extraction of the *M. charantia* whole plant. Similarly, the result in this work did not agree with the work of Abalaka *et al*. (2010). This might be due to the use of chromatographically separated pure fractions of crude extracts of whole plant of *M. charantia* before being tested for antibacterial activity. The test organisms used might have also contributed to the difference in results.

Furthermore, it was observed that the diameter of the zones of inhibition obtained from *E. coli*, *S. typhimurium and K. pneumoniae* increase as the concentration of the crude extracts increase (Tables 5-8). This result is in conformity with the work of Adegbola *et al*. (2016).

In addition, the AQE recorded the highest antibacterial activity with *E. coli* compared to other crude extracts of whole plant of *M. charantia* (Tables 5-8). This result did not agree with the work of Adegbola *et al*. (2016). This result might be due to sufficient bioactive agents in the AQE, hence, the highest antibacterial activity recorded.

However, the results of the antibacterial activity of the positive control (Ciprofloxacin) increases as the concentration used was increased from 40 mg/ml to 100 mg/ml as shown in Tables 5-8. The wideness in the zones of inhibition recorded with Ciprofloxacin in Tables 5-8 as compared to the crude extracts show the purity of the standard drug. The antibacterial activity of the crude extracts might be better if they are chromatographically separated in order to obtain the fractions as reported in the work of Abalaka *et al*. (2010). Hence, the results obtained in this study might support the use of *M. charantia* in herbal medicine and it could be used as a source of broad spectrum oral antimicrobial agent for the treatment of diseases associated with these pathogenic bacteria investigated as reported by Adegbola *et al*. (2016) and Mada *et al*. (2013). The results obtained for the negative control (DMSO) were expected since it contains no antibacterial agent (Tables 5-8).

CONCLUSION

The research work showed that there are differences in the yields of crude extracts of whole plant of *M. charantia* when different solvents are used and the aqueous extract has a better antibacterial activity than other crude extracts subjected to antibacterial activity against selected clinical isolates.

CONFLICT OF INTEREST

There is no conflict of interest among the authors.

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REFERENCES

Abalaka, M. E., Oghenerobor Benjamin Akpor, O. B. & Osemwegie, O. O. (2017). Green synthesis and antibacterial activities of silver nanoparticles against *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Advancements in Life Sciences – International Quarterly Journal of Biological Sciences*, 4 (2): 60-65.

Abalaka, M. E., Oyewole O. A. & Kolawole, A. R. (2012). Antibacterial Activities of *Azadirachta Indica* against Some Bacterial Pathogens. *Advances in Life Sciences*, 2(2): 5-8.

Abalaka, M. E., Onaolapo, J. A., Inabo, H. I. & Olonitola, O. S. (2010). Antibacterial Activity of Chromatographically Separated Pure Fractions of Whole Plant of *Momordica charantia* L (Cucurbitaceae). *Advances in Environmental Biology*, C(C): CC-CC.

Adegbola, R. A., Akinbile, Y. A. & Awotoye, J. (2016). *Mormodica charantia* Linn. A Potential Antibiotic and Anti-Fungal Drug. *International Journal of Pharmaceutical Science Invention*, 5 (2): 21-27.

Ahmad, N., Hassan, M. R. & Halder, H. (1999). Effect of *Momordica charantia* (Karolla) extracts on fasting and postprandial serum glucose levels in NIDDM patients. *Bangladesh Medical Research Council Bulletin*, 25: 11-13.

Al-Sayed, E., Martiskainen, O., Seif el-Din, S. H., Sabra, A. A., Hammam, A. O., El-Lakkany, N. M. & Abdel-Daim, M. M. (2014). Hepatoprotective and antioxidant effect of bauhinia hookeri extract against Carbon tetrachloride induced hepatotoxicity in mice and characterization of its bioactive compounds by HPLC-PDA-ESI-MS/MS. *BioMed Research International*, Article ID 245171.

Altundağ, E. & Öztürk, M. (2011). Ethnomedicinal studies on the plant resources of East Anatolia,Turkey. *Procedia Social Behavioural Science*, 19:756-777.

Bachok, M. F., Yusof, B. N., Ismail, A. & Hamid, A. A. (2014). "Effectiveness of traditional Malaysian vegetables (ulam) in modulating blood glucose levels". *Asia Pacific Journal of Clinical Nutrition*, 23(3): 669-76.

Beckman Coulter Incorporation (2016). GenomeLab™ Dye Terminator Cycle Sequencing and DNA Clean Up for Sequencing Reaction and Protocol. 608019 BA (pp.1-4). Kraemer Blvd, Brea, USA:250 S, CA 92821. Available at *www.beckmancoulter.com*

Bioneer Incorporation (2012). Accuprep Genomic DNA Extraction Protocol and Accupower Hotstart PCR Protocol. Revision 0 (pp. 1-10). Alameda, USA: 1000 Atlantic Avenue, CA 94501. Available at *www.bioneer.com*.

Cheesbrough, M. (2010). *District Laboratory Practice in Tropical Countries in* Laboratory Manual (pp. 146-157). UK: Cambridge University Press.

Clinical Laboratory Standards Institute (2015). *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement*. CLSI document M100-S25, Volume 35, No. 3.950 West Valley Road, Suite 2500 Wayne, Pennsylvania 19087, USA.

Evans, W. C. (2002). Trease and Evans Pharmacognosy15th Edition (pp. 42-44. 214-314, 221-229, 246-249, 304-306, 331-332, 391-393). London, U.K: Saunders Publishers.

Ewansiha, J. U., Garba, S. A., Musa, G., Daniyan, S. Y., Busari, M. B., Innalegwu, D. A. & Doughari, J. H. (2016). Preliminary Phytochemical and Antimicrobial Activity of Citrus x limon (L) Burm. f. (lemon) Leaf Extract against Some Pathogenic Microorganisms. *Journal of Applied Life Sciences International*, 6(4): 1-10.

Ghasemzadeh, A., Jaafar, H. Z. E. & Rahmat, A. (2010). Antioxidant activities, total Phenolics and flavonoids content in two varieties of Malaysia Young Ginger (*Zingiber officinale Roscoe*). *Molecules*, 15: 4324-4333.

Helal, M. S., Youssef, F. M., Moursi, M. K., Khalil, W. F. & Abdel-Daim, M. M. (2015). Effectiveness of prebiotic as an alternative to the antimicrobial growth promoter on growth performance, blood constituents, intestinal healthiness and immunity of broilers. *AJVS*, 45(1): 13-25.

Jayasooriya, A. P., Sakono, M. & Yukizaki, C. (2000): Effects of *M. charantia* powder on serum glucose levels and various lipid parameters in rats fed with cholesterol-free and cholesterol-enriched diets, *Journal of Enthnopharmacology,* 72: 331-336.

Leatherdale, B. A., Panesar, R. K. & Singh, G. (2001). Improvement in glucose tolerance due to *Momordica charantia* (karela). *British Medical Journal (Clin ResEd)*, 282: 1823-1824.

Li, X. & Krumholz, L. R. (2007). Regulation of asenate resistance in *Desulfovibrio desulfuricans* G20 by an *ars*RBCC operon and an *ars*C Gene. *Journal of Bacteriol*ogy, 189 (10): 3705-11.

Mada, S. B., Garba, A., Mohammed, H. A., Muhammad, A., Olagunju, A. & Muhammad, A. B. (2013). Antimicrobial activity and phytochemical screening of aqueous and ethanol extracts of *Momordica charantia* L. leaves. *Journal of Medicinal Plants Research*, 7(10): 579-586.

Mamta, S., Jyoti, S., Rajeev, N., Dharmendra, S. & Abhishek, G. (2013). *Journal of Pharmacognosy and Phytochemistry*, 1 (6): 168-180.

Murray, P. R., Baron, E. J., Jorgensen, J. H., Landry, M. L. & Pfaller, M. A. (2007). Manual of Clinical Microbiology. Ninth Edition. Washington DC: ASM Press.

National Centre for Biotechnology and Information (NCBI) (1988). Sequence BLAST. Available at *www.ncbi.nlm.nih.gov*.

Neumann, U. P., Berg, T., Baha, M., Puhl, G., Guckelbeger, O., Langreh, J. M. & Neuhaus, P. (2004). Long-term outcome of liver transplant for hepatitis C. A 10 year's follow- up. *Transplantation* 77(2): 226-231.

Oke, F. & Aslim, B. (2010). Biological potentials and cytotoxicity of various extracts from endemic *Origanum minutiflorum* O. Schwarz & P.H. Davis. *Food and Chemical Toxicology*, 48: 1728- 1733.

Oyama, M. O., Malachi, O. I. & Oladejo, A. A. (2016). Phytochemical Screening and Antimicrobial Activity of Leaf Extract of *Jatropha curcas*. *Journal of Advances in Medical and Pharmaceutical Sciences*, 8(1): 1-6.

Quinlan, M. B., Quinlan, R. J. & Nolan, J. M. (2000). Ethnophysiology and herbal treatments of intestinal worms in Dominica, West Indies. *J. Ethanopharmacol*. 80: 75-83.

Silva, E. M., Souza, J. N. S., Rogez, H., Rees, J. F. & Larondelle, Y. (2007). Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. *Food Chemistry*, 101: 1012-18.

Srivastava, J., Lambert, J. & Vietmeyer, N. (1996). Medicinal plants: An expanding role in development. World Bank Technical Paper No. 320.

Taylor, L. (2000). Technical Data Report for Bitter Melon (*Momordica charantia*). Herbal Secrets of the Rainforests 2nd ed. Sage Press Inc.

Ukoha, P. O., Cemaluk, E. A. C., Nnamdi, O. L. & Madus, E. P. (2011). Tannins and other phytochemical of the *Samanaea saman* pods and their antimicrobial activities. *African Journal of Pure and Applied Chemistry*, 5(8): 237-244.

Welhinda, J., Karunanayake, E. H. & Sheriff, M. H. (2002). Effect of *Momordica charantia* on the glucose tolerance in maturity onset diabetes. *Journal of Ethnopharmacology*, 17: 277-282.

World Health Organization (WHO) (2003). Traditional Medicine. Fact sheet No. 134.

Yankova, T. (2005). Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *Journal of Ethnopharmacology*. 96: 145-150.

Yeşilada, E., Sezik, E., Honda, G., Takaishi, Y., Takeda, Y. & Tanaka, T. (1999). Traditional medicine in Turkey IX: Folk medicine in Northwest Anatolia. *Journal of Ethnopharmacology*, 64: 195-10.

Zhu, Z. J., Zong, Z. C., Luo, Z. Y. & Xiao, Z. Y. (1990). Studies on the active constituent of *Momordica charantia*, 25: 893-903.