

ANTIMICROBIAL AND PHYTOCHEMICAL PROPERTIES OF CRUDE EXTRACTS OF SOME SELECTED PLANTS AGAINST SOME MICROBIAL ISOLATES

Chukwudebe¹, E.P., Bankole, S.O^{*2}, Asonibare, A.O² and Adedokun, S.A³

¹Biomedical Research Centre, Forestry Research Institute of Nigeria, Jericho, Ibadan.

²Biotechnology Section, Bioscience Department

³Basic Science & General Studies Department, Federal College of Forestry, Ibadan

<http://doi.org/10.35410/IJAEB.2019.4428>

ABSTRACT

Antimicrobial and phytochemical properties of the crude extract of some selected plant samples against some microbial isolates was investigated. Plant samples were extracted using ethyl acetate and methanol as extracting solvents based on polarity. Antimicrobial studies was carried out using Agar disc diffusion method. Zones of inhibitions were measured, Tetracycline, Ampicillin, Chloramphenicol and Ofloxacin antibiotics were used as positive control. Extracting solvent served as negative control and work was done in triplicates. Plant samples were found to contain several bioactive compounds in various combinations. Ethyl acetate Pawpaw leaves extract gave the best results with zones of inhibition as high as $27.3 \pm 11.7\text{mm}$ and $25.3 \pm 0.6\text{mm}$ against *Escherichia coli* and *Candida albicans* respectively, while methanol Pawpaw leaves extracts gave the best result with zone of inhibition as high as $25.0 \pm 13.0\text{mm}$ against *Staphylococcus aureus*. Ampicillin and Chloramphenicol in all cases and for both extracting solvents showed no significant difference in the mean zone of inhibition compared with plant extracts at 5% level of significance. The minimum bactericidal concentration was found with ethyl acetate pawpaw leaves extract against *Escherichia coli* and *Pseudomonas aeruginosa* (bactericidal) at 100mg/ml and 200mg/ml respectively while all the others were bacteriostatic.

It was thus concluded that the selected plant extracts could serve as alternative drugs for the treatment of diseases.

Keywords: Bacteriostatic, Extract, Inhibition, Phytochemical.

1. INTRODUCTION

Indigenous knowledge of herbal medicine is a big source of the modern knowledge (Kakare^{et al.}, 2012). Herbal plants and their preparations have been reported for antimicrobial, antimalarial, anti-inflammatory, anti-parasitic, anti-diabetic, anthelmintic, anti-obesity, anti-cancer and anti-viral activities (Nimri^{et al.}, 1999). *Caricapapaya* Linn (Pawpaw), belonging to family *Caricaceae* is commonly known as papaya in English is commonly known for its food and nutritional values throughout the world. The medicinal properties of papaya fruit and other parts of the plant are also well known in traditional system of medicine. Each part of papaya tree possesses economic value when it is grown on a commercial scale (Krishna ^{et al.}, 2008). Traditionally, leaves have

been used for treatment of a wide range of ailments, like in treatment of malaria, dengue, and jaundice, immune modulatory and antiviral activity amongst others. According to Ayoola and Adeyeye (2010), the phytochemical analysis of the leaves of *Carica papaya* showed the presence of saponins, cardiac glycosides, and alkaloids. Tannin was absent in the leaves. The presence of saponins supports the fact that pawpaw leaf has cytotoxic effects such as permeabilization of the intestine, as saponins are cytotoxic (Okwu and Okwu, 2004). Vijayakumar *et al.*, (2015) in their study using the extracts prepared from leaf (acetone, aqueous, ethanol and methanol) of *Carica papaya*, found the presence of Alkaloids, Flavonoids, phenols, Saponins, and Sterols, also it exhibited highest antimicrobial activity against pathogenic microbes bacteria and fungi.

Yogiraj *et al.*, (2014) in their report on the antibacterial and antifungal ability of both fresh and dried leaves of *Carica papaya* against bacteria and fungi of medical importance using the disc diffusion method reported very significant broad spectrum antimicrobial activity against Gram-negative and Gram-positive bacteria and fungi.

Mangifera indica (Mango) is a large evergreen tree, with a heavy, dome-shaped crown. It belongs to the family *Anacardiaceae*. It is found all over the tropical regions of the world where it is used as a horticultural and medicinal plant. Various parts of the plant are used as a dentifrice, antiseptic, astringent, diaphoretic, stomachic, vermifuge, tonic, laxative and diuretic and to treat diarrhea, dysentery, anaemia, asthma, bronchitis, cough, hypertension, insomnia, rheumatism, toothache, leucorrhoea, haemorrhage and pile. The leaves have been reported to contain saponins, glycosides, unsaturated sterols, polyphenols, euxanthin acid, mangiferine, mangin, gallic tannins, etc. The ashes of the leaves are used to treat burns, scalds, sores, cough and diarrhoea in South America and other parts of the world (Dweck, 2001; Hirte, 2002). According to Okwu and Ezenagu (2008), in their evaluation of the phytochemical composition of mango (*Mangifera indica*), the stem, bark and leaves showed presence of tannins, saponins, alkaloids and flavonoids. According to Nwankwo and Osaro-Mathew (2014), it was stated that the ethanolic extract of *M. indica* had mild inhibitory effects on *Staphylococcus aureus* and *Escherichia coli* while the hot water extract of the same concentration showed no inhibitory effects on these organisms. Mary *et al.*, (2013) in her work on phytochemical analysis and anticancer activity of leaf extract of *mangifera indica* stated that it had antimicrobial activity against nine bacteria. According to the research, the presence of phyto-constituents in the leaf extracts may be responsible for these antibacterial activity of the plant.

2. MATERIALS AND METHODS

Collection of Plant Parts: Leaves, as well as Ripe and Unripe fruit peel of *Mangifera indica* (Mango) and *Carica papaya* (paw paw) were collected from FRIN Herbal garden. They were identified and authenticated at the taxonomy department of the institute. Samples collected were allowed to dry at room temperature (35°C) before milling into powdered form. They were kept in tight containers for further use.

Collection and Isolation of Test Microorganisms: Clinical isolates were collected. The four isolates used in this study were *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Pseudomonas aeruginosa*. These were obtained from University of Ilorin teaching Hospital, Kwara State, Ilorin. They were sub-cultured and maintained in Nutrient agar and Potato dextrose agar slant for bacteria and fungi respectively.

Preparation of Inoculum: A loopful of test organism was taken from agar slants into test tubes containing nutrient broth and Potato dextrose liquid. The test tubes was then incubated for 24hours at 37°C and 48hours at 30°C. The isolates in the broth was standardized using 0.5ml Mcfarlands turbidity standard. This was done by adding 0.5ml of 1.175% of anhydrous Barium chloride drop-wise to 99.5ml of 1% sulphuric acid in 100ml volumetric flask and constantly swirling. This was mixed for 3-5 minutes until solution appeared homogeneous and free of clumps. Optical density of solution was examined using a spectrophotometer and wavelength of 625nm was taken which is the accepted range for Mcfarland 0.5ml. This was then dispensed into a glass screw cap tube and sealed with paraffin and then stored at room temperature until needed.

Extraction of Plant Materials: 200grams of milled plant parts were soaked in 1000ml of ethylacetate (extracting solvent). This was stirred with a glass rod and left in the shaker for 12hours. It was then filtered using whatsmann filter paper. The solution was then passed through the Rotary evaporator. Filtrate was left in the dessicator for future use. Similar procedure was also employed using methanol as extracting solvent.

Phytochemical Screening of Various Extracts of Plant Sample: This will be performed using the method described by Nweze *et al.*, (2004) and Senthilkumar and Reetha (2009). The samples were screened for cardiac glycosides, alkaloids, flavonoids, triterpenes and steroids, anthocyanin and betacyanin, phenols, Tannins, saponin, glycosides, and anthraquinones.

Antimicrobial Sensitivity testing of the Extract on the Clinical isolates: Using agar disc diffusion techniques as described by Kirby-Bauer technique (Bauer *et al.*, 1966; John and James, 1999), various concentration of plant extract (400mg/ml, 200mg/ml, 100mg/ml and 50mg/ml) were used. Paper discs of 0.3 diameter in size were impregnated with the selected plant extracts. It was left to dry for 10minutes, before dispensing unto the surface of the already inoculated Muller Hinton Agar /Sabouraud Dextrose Agar plate and allowed to diffuse for half an hour at 40°C on the surface of the medium. The plates were incubated at 37°C for 24 hours and the zone of inhibition around each disc was measured for sensitivity, mild sensitivity or resistance. Diameters of the inhibition zones were measured using a metre rule. The antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the plant extract. Each assay was carried out in triplicates under strict aseptic conditions. The absence of zone inhibition was interpreted as the absence of activity (Kohner *et al.*, 1994; Mathabe *et al.*, 2006). The activities are expressed as resistant, if the zone of inhibition was less than 7 mm, intermediate or mildly sensitive (8-10 mm) and sensitive if more than 11 mm (Assam *et al.*, 2010). Commercially prepared antibiotics discs (Tetracycline, Ampicillin, Chloramphenicol and Ofloxacin) were used to determine the drug sensitivity and resistance pattern of bacteria. These discs were placed on the plates inoculated with different strains. These served as positive control. Disc containing extracting solvents served as negative control. Similar to this, fungicidal effect of the plant extract was assessed by the inhibition of mycelia growth of the fungus and was observed as a zone of inhibition near the disc. The antifungal effect was seen as crescent shaped zones of inhibition. Nystatin (standard antifungal agent) also served as its positive control.

Minimum Inhibitory Concentrations (MIC) and Minimum Bacteriocidal Concentration (MBC): This was carried out by broth dilution method and plating method according to European Society of Clinical Microbiology and Infectious Diseases (ESCMID, 2003) and

(Andrews, 2006). Different concentrations of ethyl acetate and methanol extracts of leaves and peels of *Carica papaya* (Pawpaw) and *Mangifera indica* (Mango) from 400mg/ml to 6.25mg/ml (400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml) were prepared using extracting solvent following a two-fold dilution. Standardized test organisms in broth culture (Muiller Histone broth and Sabouraud Dextrose broth) were used. About 9ml of broth culture together with 0.5ml of standardized organism and 0.5ml of extracting solvent was placed in test tubes and incubated for 24 hours at 35⁰C, the presence of turbidity indicated growth of organism while tube with less concentration, having no turbidity was recorded as MIC. This was done in triplicate. After determination of MIC, pour plate method was used to grow the test tubes positive to MIC. Plates were incubated for 24 hours at 35⁰C. Plates showing no growth of organisms were taken as the MBC. Ampicillin and Nystatin served as positive control while the last column of test tubes was left blank (that is without extract or antibiotics). This served as negative control.

3. RESULTS AND DISCUSSION

Qualitative Phytochemical Analysis

Table 1 shows the result obtained from the phytochemical screening of crude plant extract performed. It was observed that alkaloid, flavonoid, tannins were found present in ripe mango peel. Steroid was found to be present in mango leaves, unripe pawpaw peel and pawpaw leaves while phenol was found to be present only in mango leaves. Also mango leaves was found to contain all the compounds tested for (steroid, alkaloid, flavonoid, tannins, saponins, glycosides, anthraquinones, cardiac glycosides and phenol). Pawpaw leaves was also found to contain all compound except glycoside and phenol. Ripe pawpaw peel was found to contain only alkaloid and saponins while unripe pawpaw peel contained steroid, tannins and saponins. Also, Unripermango peel contained only alkaloid, flavonoids and saponins.

Table1: Qualitative Phytochemical screening of Plant Crude Extracts

Bioactive Compounds	Plant Extracts					
	Ripe mango peel	Unripe mango peel	Mango leaves	Ripe pawpaw peel	Unripe pawpaw peel	Pawpaw leaves
Steroid	-	-	+	-	+	+
Alkaloid	+	+	+	+	-	+
Flavonoid	+	+	+	-	+	+
Tannins	+	-	+	-	-	+
Saponin	-	+	+	+	+	+
Glycosides	-	-	+	-	-	-

Anthraquinones	-	-	+	-	-	+
Cardiac glycosides	-	-	+	-	-	+
Phenol	-	-	+	-	-	-

Antimicrobial Activities of Plant Extracts on Selected Organism

It was observed that ethyl acetate ripe mango peel extract was more active against *Candida albicans* with highest zone of inhibition of 18.3 ± 5.1 mm while lowest zone of inhibition was observed with *Pseudomonas aeruginosa* at 10.0 ± 2.6 mm. Also, methanol Ripe mango peel extract had highest zone of inhibition against *Escherichia coli* at 20.7 ± 2.5 mm while lowest zone of inhibition was observed against *Candida albicans* at 10.3 ± 3.8 mm.

It was observed that ethyl acetate Unripe mango peel extract was more active against *Escherichia coli* with highest zone of inhibition of 21.7 ± 2.1 mm while lowest zone of inhibition was observed with *Pseudomonas aeruginosa* at 11.3 ± 1.5 mm. Also, methanol Unripe mango peel extract had highest zone of inhibition against *Staphylococcus aureus* at 22.3 ± 3.2 mm while lowest zone of inhibition was observed with *Candida albicans* at 10.3 ± 5.0 mm.

It was observed that both ethyl acetate and methanol Mango leaves extract were active against *Staphylococcus aureus* with highest zone of inhibition of 22.3 ± 1.2 mm and 23.7 ± 2.1 mm respectively while lowest zone of inhibition for ethyl acetate Mango leaves was observed against *Escherichia coli* at 11.0 ± 1.7 mm and for methanol Mango leaves extract, it was observed to have lower zone of inhibition against *Candida albicans* at 15.3 ± 12.5 mm.

It was observed that both ethyl acetate and methanol Ripe Pawpaw peel extract were active against *Staphylococcus aureus* with highest zone of inhibition of 14.3 ± 9.3 mm and 22.7 ± 7.2 mm respectively. Lowest zone of inhibition for ethyl acetate Ripe Pawpaw peel was observed against *Escherichia coli* at 10.0 ± 4.4 mm. Also for methanol Ripe Pawpaw peel extract was observed to have lower zone of inhibition against *Candida albicans* at 14.7 ± 9.3 mm.

It was observed that ethyl acetate Unripe Pawpaw peel extract was more active against *Pseudomonas aeruginosa* with highest zone of inhibition of 21.7 ± 3.1 mm while its lowest zone of inhibition was observed against *Candida albicans* at 10.0 ± 2.0 mm. Also, methanol Unripe Pawpaw peel extract had highest zone of inhibition against *Staphylococcus aureus* at 19.0 ± 6.1 mm while its lowest zone of inhibition was observed against *Candida albicans* at 10.7 ± 3.1 mm.

It was observed that ethyl acetate Pawpaw leaves extract was more active against *Escherichia coli* with highest zone of inhibition of 27.3 ± 11.0 mm while its lowest zones of inhibition was against *Staphylococcus aureus* at 18.7 ± 3.2 mm. Methanol Pawpaw leaves extract had highest zone of inhibition against *Staphylococcus aureus* at 25.0 ± 13.0 mm while lowest zones of

inhibition was observed against *Escherichia coli* and *Candida albicans* at $12.0 \pm 10.6\text{mm}$ and $12.0 \pm 10.4\text{mm}$ respectively

Furthermore, the varied concentrations (200mg/ml) of ethyl acetate Unripe mango peel, gave zones of inhibition sensitive against *Staphylococcus aureus* at 12.7 ± 4.5 and *Candida albicans* at 12.3 ± 7.4 . Also, mango leaves showed sensitive zones of inhibition against *Staphylococcus aureus* at 15.3 ± 0.6 and *Candida albicans* at 11.0 ± 4.6 , and pawpaw leaves showed sensitive zones of inhibition against all four organisms (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*) at 12.7 ± 2.1 , 10.7 ± 1.2 , 16.7 ± 1.5 and 14.0 ± 2.6 respectively. All other concentrations showed intermediate to non-sensitive zones of inhibition. Whereas, varied concentrations (200mg/ml) of methanol extract ripe mango peel gave sensitive zones of inhibition against *Escherichia coli* at 13.7 ± 5.0 , unripe mango peel showed sensitive zones of inhibition against *Escherichia coli* and *Staphylococcus aureus*, at 13.3 ± 5.5 and 13.0 ± 5.6 respectively, mango leaves showed sensitive zones of inhibition against *Escherichia coli* and *Staphylococcus aureus*, at 16.7 ± 4.2 and 14.3 ± 4.7 respectively, ripe pawpaw peel showed sensitive zones of inhibition against *Staphylococcus aureus*, at 12.3 ± 4.5 and pawpaw leaves showed sensitive zones of inhibition against *Staphylococcus aureus*, at 15.7 ± 6.0 .

Plant Extract	Organism	Concentration(mg/ml)/ Average Zone of Inhibition(mm)							
		Ethyl acetate				Methanol			
		400	200	100	50	400	200	100	50
RIPE MANGO PEEL	<i>Escherichia coli</i>	13.7±4.9	-	-	-	20.7±2.5	13.7±5.0	10.7±4.2	6.3±2.1
	<i>Staphylococcus aureus</i>	17.3±3.1	9.3±1.5	3.0±1.7	2.0±1.0	22.0±5.3	9.7±7.7	9.7±2.9	4.7±1.5
	<i>Pseudomonas aeruginosa</i>	10.0±2.6	-	-	-	13.7±3.1	-	-	-
	<i>Candida albicans</i>	18.3±5.1	9.3±3.5	6.0±1.0	2.7±0.6	10.3±3.8	-	-	-

UNRIPE MANGO PEEL	<i>Escherichia coli</i>	21.7±2.1	10.0±1.0	6.0±1.0	2.3±1.2	21.3±3.1	13.3±5.5	8.7±3.8	4.3±3.5
	<i>Staphylococcus aureus</i>	20.0±2.6	12.7±4.5	6.3±0.6	3.3±0.6	22.3±3.2	13.0±5.6	7.0±2.0	1.3±1.5
	<i>Pseudomonas aeruginosa</i>	11.3±1.5	-	-	-	14.3±1.5	-	-	-
	<i>Candida albicans</i>	17.7±10.2	12.3±7.4	6.3±2.3	1.3±2.3	10.3±5.0	-	-	-
MANGO LEAVES	<i>Escherichia coli</i>	11.0±1.7	-	-	-	22.7±0.6	16.7±4.2	9.3±4.2	5.3±2.1
	<i>Staphylococcus aureus</i>	22.3±1.2	15.3±0.6	8.7±1.5	3.7±0.6	23.7±2.1	14.3±4.7	6.7±3.1	5.3±2.5
	<i>Pseudomonas aeruginosa</i>	15.7±1.5	9.0±1.0	4.0±1.0	1.3±0.5	18.3±6.7	9.7±9.5	9.3±3.2	2.0±1.7
	<i>Candida albicans</i>	18.3±5.7	11.0±4.6	5.0±4.4	1.7±0.6	15.3±12.5	6.7±6.1	4.3±3.2	1.7±2.1
RIPE PAWPAW PEEL	<i>Escherichia coli</i>	10.0±4.4	-	-	-	15.3±2.1	8.7±2.1	5.0±2.0	2.7±1.5
	<i>Staphylococcus aureus</i>	14.3±9.3	-	-	-	22.7±7.2	12.3±4.5	7.7±2.1	5.7±3.1
	<i>Pseudomonas aeruginosa</i>	10.7±9.3	-	-	-	17.7±6.0	10.3±4.0	5.3±2.5	2.7±1.5

	<i>Candida albicans</i>	13.0±11.3	-	-	-	14.7±9.3	-	-	-
UNRIPE PAWPAW PEEL	<i>Escherichia coli</i>	11.7±10.2	-	-	-	16.3±4.0	7.3±7.5	4.7±3.8	1.7±2.1
	<i>Staphylococcus aureus</i>	12.7±4.0	-	-	-	19.0±6.1	9.7±2.5	5.7±3.5	2.3±2.1
	<i>Pseudomonas aeruginosa</i>	21.7±3.1	9.0±7.3	7.7±1.2	3.7±1.5	13.3±6.7	-	-	-
	<i>Candida albicans</i>	10.0±2.0	-	-	-	10.7±3.1	-	-	-
PAWPAW LEAVES	<i>Escherichia coli</i>	27.3±11.7	12.7±2.1	7.7±2.5	-	12.0±10.6	-	-	-
	<i>Staphylococcus aureus</i>	18.7±3.2	10.7±1.2	7.3±0.6	1.7±1.2	25.0±13.0	15.7±6.0	9.3±3.2	-
	<i>Pseudomonas aeruginosa</i>	21.7±11.8	16.7±1.5	6.3±1.5	2.7±0.6	15.0±8.7	10.3±9.1	4.3±2.1	3.3±1.2
	<i>Candida albicans</i>	25.3±0.6	14.0±2.6	6.3±2.3	-	12.0±10.4	-	-	-

Antibiotics Sensitivity testing against Clinical Isolates: Using Tetracycline, Ampicillin, Ofloxacin, Chloramphenicol (antibiotics) as positive control for antibacterial analysis and Nystatin (antifungal) as positive control for antifungal analysis against isolates revealed that for *Escherichia coli*, tetracycline, ampicillin, ofloxacin and chloramphenicol gave zones of inhibition of $17.0 \pm 0.00\text{mm}$, $25.0 \pm 0.00\text{mm}$, $28.0 \pm 0.00\text{mm}$ and $20.0 \pm 0.00\text{mm}$ respectively. For *Staphylococcus aureus*, tetracycline, ampicillin, ofloxacin and chloramphenicol gave zones

of inhibition of $20.0 \pm 0.00\text{mm}$, $18.0 \pm 0.00\text{mm}$, $24.0 \pm 0.00\text{mm}$ and $23.0 \pm 0.00\text{mm}$ respectively. For *Pseudomonas aeruginosa*, tetracycline, ampicillin, ofloxacin and chloramphenicol gave zones of inhibition of $20.0 \pm 0.00\text{mm}$, $23.0 \pm 0.00\text{mm}$, $22.0 \pm 0.00\text{mm}$ and $12.0 \pm 0.00\text{mm}$ respectively. For *Candida albicans*, nystatin gave zone of inhibition of $26.0 \pm 0.00\text{mm}$. Negative control using extracting solvent was negative for both ethyl acetate and methanol.

Table 3: Antibiotics Sensitivity testing against Clinical Isolates

Organisms	Positive control (mm)					Negative control	
	Tetracycline	Ampicillin	Ofloxacin	Chloramphenicol		Ethyl	
				Nystatin	Acetate	Methanol	
<i>Escherichia coli</i>	17.0	25.0	28.0	20.0	-		
<i>Staphylococcus aureus</i>	20.0	18.0	24.0	23.0	-		
<i>Pseudomonas aeruginosa</i>	20.0	23.0	22.0	12.0	-		
<i>Candida albicans</i>	-	-	-	-	26.0		
						Negative	Negative

Minimum Inhibitory and Bactericidal Concentration of Plant Extract on Microbial Isolates

The minimum inhibitory concentration (MIC) values of Unripe mango peel and Mango leaves ethyl acetate extracts treated on *S. aureus* and Mango leaves ethyl acetate extracts against *C. albicans* was found to be 200mg/ml. On the other hand, the MIC values of Pawpaw leaves ethyl acetate extract treated on *E. coli* and *P. aeruginosa* were found to be 100mg/ml and 200mg/ml respectively. For Mango and Pawpaw leaves, methanol extract showed MIC values against *S. aureus* at 200mg/ml and 100mg/ml respectively, while Mango and Pawpaw leaves methanol extracts showed MIC values against *P. aeruginosa* at 100mg/ml respectively. Unripe mango peel was observed to have shown MIC value when used against *E. coli* at 100mg/ml. MIC values of Nystatin and ampicillin was determined on *C. albicans*, *E. coli* and *S. aureus* and showed results of 50mg/ml and 100mg/ml respectively. Negative control was left blank and therefore showed no results.

Minimum bactericidal concentration of plant extracts of unripe mango peels, mango leaves ethyl acetate extract and unripe mango peels, mango and pawpaw leaves methanol extract showed further growth of microorganisms at 200mg/ml concentration. However pawpaw leaves ethyl acetate extracts of 100mg/ml and 200mg/ml concentrations respectively against *E. coli* and *P. aeruginosa* showed no further growth on petri dish.

Table 4: Minimum Inhibitory Concentrations of Plant Extracts

Extracting Solvent	Plant Extract	Minimum Inhibitory Concentrations (mg/ml)			
		<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
Ethyl Acetate	UMP	-	200	-	-
	ML	-	200	-	200
	PL	100	-	200	-
Methanol	UMP	200	-	-	-
	ML	-	200	100	-
	PL	-	100	100	-
Nystatin (positive control)		-	-	-	50
Ampicillin (positive control)		100	100	-	-

UMP- Unripe mango peel, ML- Mango leaves, PL- Pawpaw leaves

4.DISCUSSION

Phytochemical screening: The results obtained from this study which carried out an analysis of the qualitative phytochemical properties of crude extracts of some selected plant samples against some microbial isolates using six plant samples (Mango leaves, Ripe and Unripe mango peels, Pawpaw leaves, and Ripe and Unripe pawpaw peels) showed the presence of phytochemical properties. Similar findings were made by Ayoola and Adeyeye (2010) who in their findings on the phytochemical properties of the leaves of *Carica papaya* showed the presence of saponins, cardiac glycosides, and alkaloids. It is also in agreement with the findings of Okwu and Ezenagu (2008), Aravind *et al.*, (2013) and Vijayakumar *et al.*, (2015). The presence of saponins according to Okwu and Okwu. (2004), indicates the cytotoxic effects of the plant extracts. The presence of the bioactive components in pawpaw and mango leaves extract according to Mary *et al.*, (2013) and Aravind *et al.*, (2013), could be said to be the reasons of its effectiveness against microorganisms.

Antimicrobial activities of plant extracts on selected isolates: According to Bacon *et al.*, (2017), the higher the concentration of extract to solvent the more the zone of inhibition. This therefore supports the reason for the observed higher zones of inhibition in higher concentrations in this research. Zones of inhibition ≤ 7 mm indicated that the organism was resistant, 8-10mm indicated an intermediate sensitivity, while ≥ 11 mm indicated sensitivity. Sensitivity implied that the plant

extract could inhibit the growth of that particular organism at the given level of concentration. 400mg/ml and 200mg/ml concentrations gave the best results in both extracting solvent. For ethyl acetate extracts, Pawpaw leaves gave the best results with highest zones of inhibition as can be observed in table 2 against *Escherichia coli* and *Candida albicans* respectively. Meanwhile, in methanol extracts, Pawpaw leaves gave the best result with highest zone of inhibition against *Staphylococcus aureus*. Mango leaves was also observed to have given the high zones of inhibition against *Staphylococcus aureus* as seen in Table 2. This result shows that pawpaw and mango leaves plant extract is active against both gram positive and gram negative bacteria therefore is at disparity with an earlier report by Jigna and Chanda (2006), who in his findings indicated that plant extracts were more active against gram-positive bacteria than gram-negative bacteria.

Antibiotic sensitivity testing had zones of inhibition equivalent to that of plant part extracts. This therefore suggests that plant parts especially pawpaw and mango leaves extract are as good as other commercially sold antibiotics in inhibiting these microorganisms and therefore could possibly serve as an alternative. Pawpaw leaves had no further growth and was also taken as the minimal bactericidal concentration.

CONCLUSION

Based on the findings from the study it can be concluded that: Plant samples contained several bioactive compounds (steroid, alkaloid, flavonoid, tannins, saponins, glycosides, anthraquinones, cardiac glycosides and phenol) which accounted for the activities of plant against microorganisms. The minimum bactericidal concentration was found with ethyl acetate pawpaw leaves extract against *Escherichia coli* and *Pseudomonas aeruginosa* (bactericidal) while all the others were bacteriostatic. Pawpaw leaves was found to have the highest zone of inhibition and could therefore be said to be the best plant extract against the selected clinical bacterial isolates.

REFERENCES

- Alonso-Paz, E., Cerdeiras, M.P., Fernandez, J., Ferreira, F., Moyna, P., Soubes, M., Vazquez, A., Veros, S. and Zunno, L. (1995). Screening of Uruguayan medicinal plants for antimicrobial activity. *J. Ethnopharmacology*.45: 67-70.
- Anesini, E. and Perez, C. (1993). Screening of plants used in Argentine folk medicine for antimicrobial activity. *J. Ethnopharmacol*.39: 119-128.
- Assam, J.P., Dzoyem, J.P., Pieme, C.A. and Penlap, V.B. (2010). In vitro antibacterial activity and acute toxicity studies of aqueous-methanol extract of *Sidarhombifolia* Linn. (Malvaceae). *Journal of the International Society for Complementary Medicine Research (ISCMR)* 10:40
- Bacon, K., Boyer, R., Denbow, C., O'keefe, S., Neilson, A. and Williams, R. (2017). Evaluation of different solvents to extract antibacterial compounds. *Food SciNutr*. 5: 497-530.
- Barreto, J.C., Trevisan, M.T. and Hull, W. E. (2008). Characterization and quantitation of polyphenolic compounds in bark, kernel, leaves, and peel of mango (*Mangifera indica* L.). *J. Agric. Food Chem*.56(14): 5599-5610.

Bruna, E.P., Fernandes, B., Borges, A.C., Almeida, J. and Barros, N.F. (1989). Effects of Eucalyptus litter extracts on microbial growth. *Pesq. Agrop. Bras.*24: 1523-1528.

Carvalho, V., Melo, V.M., Aguiar, A. and Matos, F.S. (1988). Toxicity evaluation of medicinal plant extracts by the brine shrimp (*Arthenussalina Leah*) bioassay. *Ciência e Cultura*40: 1109-1111.

Cohen, M.L. (1992). Epidemiology of drug resistance. In Implications for a post-antimicrobial era. *Science*.257: 1050-1055.

Cruz, F.G., Roque, N.F., Giesbrecht, A.M.andDavino, S.C. (1996). Antibiotic activity of diterpenes from *Mikaniatriangularis*. *Fitoterapia*. 67:189-190.

Donatus, E. O. and Vitus, E. (2008). Evaluation of the phytochemical composition of mango (*mangiferaindicalinn*) stem bark and leaves. *Int. J. Chem. Sci.*6(2): 705-716

Kohner, P.C., Rosenblatt, J.E. and Cockerill, F.R. (1994). Comparison of agar dilution, broth dilution and disk diffusion testing of Ampicillin against *Haemophilus spp.* by using in house and commercially prepared media. *J. Clin. Microbiol.* 32: 1594 -96.

Lemos, T.L.G., Monte, F.J.Q., Matos, F.J.A., Alencar, J.W., Craveiro, A.A., Barbosa, R.C.S.B. and Lima, E.D. (1992). Chemical composition and antimicrobial activity of essential oils from Brazilian plants. *Fitoterapia*.63: 266-268.

Martinez, M.J., Betancourt, J., Alonso-Gonzalez, N. and Jauregui, A. (1996). Screening of some Cuban medicinal plants for antimicrobial activity. *J. Ethnopharmacol.*52: 171-174.

Martinez, M.J., Vasquez, S.M., Espinosa-Perez, C., Dias, M. and Herrera-Sanchez, M.(1994). Antimicrobial properties of *Argentatine A* isolated from *Partheniumargentatum*. *Fitoterapia*.65: 371-372.

Martins, E. (2014).The growing use of herbal medicines: issues relating to adverse reaction and challenges in monitoring safety. *FrontiersinPharmacology*. 4: 170.

Mary- Helen, P.A., Aswathy, M.R., Deepthi, K.G., RathiMol, R., Jaison, J. J. and Jaya, S. S. (2013). *Phytochemical analysis and anticancer activity of leaf extract of mangiferaindica (kottukonamvarika).**International Journal Of Pharmaceutical Sciences And Research*. Pp823-828.

Mathabe, M.C., Nikolova, R.V., Lall, N. and Nyazema, N.Z.(2006). Antibacterial activities of medicinal plants used for the treatment of diarrhea in Limpopo Province, South Africa. *JournalofEthnopharmacology*.105:286-293.

Matos, F.J.A., Aguiar, L.M.B.A. and Silva, M.G.A. (1988). Chemical constituents and antimicrobial activity of *VataireamacrocarpaDucke*. *ActaAmazonica*. 18: 351-352.

Nascimento, S.C., Chiappeta, A. and Lima, R.M.O.C. (1990). Antimicrobial and cytotoxic activities in plants from Pernambuco, Brazil. *Fitoterapia*.61:353-355.

Nunez-Selles, J. (2005). Antioxidant Therapy; Myth or Reality. *J. Braz. Chem. Soc.*16(4): 101 – 108.

Nwankwo, I.U. and Osaro-Mathew, R.C. (2014). Assessment of the phytochemical components of *Mangifera indica*(leaf) and *Musa paradisiaca* (roots) extracts and their antibacterial activity against some common pathogenic bacteria. *Journal of Pharmacy and Biological Sciences.* 9: 08-11

Nweze, L.A., Okafor, J.L. and Nwoku, O. (2004). Antimicrobial Activities of Methanolic extracts of *Tremeguienees* (Schumm and thom) and *Morindalucida*Benth used in Nigeria traditional herbal medicinal practice. *Biol. Res.* 2: 33-48.

Santos Filho, D., Sarti, S.J., Bastos, J.K., LeitãoFilho, H.F., Machado, J.O., Araujo, M.L.C., Lopes, W.D. and Abreu, J.E. (1990). Atividadeantibacteriana de extratosvegetais. *Rev. Cien. Farm.*12: 39-46.

Sanusi, B. M., Auwalu, G., Aliyu, M., Aminu, M. and David, O.A. (2012). Phytochemical Screening and Antimicrobial Efficacy of Aqueous and Methanolic Extract of *Mangifera indica* (Mango Stem Bark). *World J Life Sci. and Medical Research.* 2(2): 81-85

Senthilkumar, P.K. and Reetha, D. (2009). Regular article Screening of antimicrobial properties of certain Indian medicinal plants. *Journal of Phytology.* 1(3): 193–198

Sofowora, A.O. (1993). Medicinal Plants and Traditional Medicine in Africa. University of Ife Press 2nd Ed. Pp 320.

Subramanian, G., Brij, B.T., Rekha, G. (2014). Antimicrobial properties of *Carica papaya*(papaya) different leaf extract against *E. coli*, *S. aureus*, *C. albicans*. *American Journal of Pharmacology and Pharmacotherapeutics.* 1: 25-39.