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BIOASSAY DIRECTED FRACTIONATION OF ANTIBACTERIAL COMPOUNDS FROM TRAVELLER'S TREE (*RAVENALA MADAGASCARIENSIS* SONNERAT) AND ITS PHYTOCHEMICAL CONSTITUENTS

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Abstract: Investigations were conducted to determine the antibacterial and phytochemical activities of extracts from the leaves of traveller's tree (*Ravenala madagascariensis*). Different concentrations (i.e. 25 - 200 mg/ml) of the extracts prepared using ethanol; n-Hexane, hot water and cold water were tested against some selected human pathogenic bacteria using agar well diffusion method. Clinical isolates and typed cultures of the organisms were screened with the plant extracts. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts to susceptible organisms were determined. The rate of inhibition of bacterial growth was also investigated using turbidometry method. Crude extracts were screened for the type and amounts of phytochemicals, while the biologically active fractions of the extracts were structurally elucidated using Infra-Red spectroscopy. Ethanol extract induced the highest zone of inhibition (10.80 ± 0.04mm) on *Proteus mirabilis* (ATCC 25933) and the lowest zone of inhibition (4.6 ± 0.01mm) on *Proteus vulgaris* when exposed to crude ethanol extract. Ethanol extract remarkably suppressed the growth of *Proteus vulgaris, Serratia marcescens, Klebsiella pneumoniae, Citrobacter freundii, Proteus mirabilis* (ATCC 25933)*, Serratia marcescens* (ATCC 39006)*, Klebsiella pneumoniae* (ATCC 13883)*, Citrobacter freundii* and *Shigella flexneri* (ATCC 12022). N-Hexane extract was only effective against *Serratia marcescens* and *Klebsiella pneumoniae* with 24% inhibition respectively. However, cold and hot water extracts had no effect on any of the test organisms, irrespective of the concentration. The clinical isolates were more susceptible to the extracts than the typed cultures in ratio 4:3. Phytochemical screening revealed the presence of tannins (0.31%), flavonoids (16.32%), phlobatannins (0.36%), cardiac glycosides (0.08%), cyanogenic glycosides (47%) and anthraquinones (16.20%) in the plant extracts.

Key Words: Antibacterial, Phytochemical, Minimum inhibitory concentration, Minimum bactericidal concentration, Zone of inhibition.

INTRODUCTION

Nigeria is blessed with thousands of household formulations to treat almost all major and minor health problems in day-to-day life. Since ancient times, diseases are treated with several medicinal plants or their extracts based on their folkloric claims. Herbal medicines usually consist of several active constituents, each of which balances the action of another (Bimal *et al.*, 2003; Fadipe and Onifade, 2015). *Ravenala madagascariensis* Sonn of the family Sterilitziaceae and commonly called as traveller's tree, is a native of Madagascar (South Africa), but often found cultivated in Indian gardens. The leaves of *Ravenala madagascariensis* have close resemblance with the leaves of *Musa paradisiaca* (Sowmayanath, 2008). It is a palm-like tree with simple alternate leaves forming a fan-like crown. It has been given the name "traveller's palm" because the sheaths of the stems hold rainwater, which supposedly could be used as an emergency drinking supply for needy travelers. Another plausible reason for its name is that the fan tends to grow in an east-west line, providing a crude compass.

Therefore, it is of great interest to screen this plant to validate its use in traditional medicine and to reveal the active principles by isolation and characterization of their constituents. Systematic screening of this plant may result in the discovery of novel active compounds. This study was designed to: determine the *in vitro* effects of the leaf extracts of traveller's tree on selected human pathogenic bacteria; investigate the effect of temperature, extraction solvent and extract concentration on the antimicrobial efficacy of the leaf extracts; evaluate the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts; obtain different fractions of the biologically active components using chromatographic techniques and determine the chemical composition of the active fractions using Infra-Red spectroscopy.

MATERIALS AND METHODS

Collection, Identification and Preparation of plant materials

Fresh leaves of traveller's tree (*Ravenala madagascariensis* Sonn.) used for this experiment were collected from Akure in Ondo state, Nigeria between May and August, 2012 using clean scalpel. The identity of the plant sample was authenticated at the Herbarium of the University of Ibadan, Nigeria and voucher specimens were deposited in the Herbarium of the Federal University of Technology Akure. The leaf samples were air-dried at room temperature for four weeks and later milled into powder using a grinding machine (Das *et al.,* 2005). The powdered sample was stored in a sterile polythene bag and kept at 28±2°C for 4 days for subsequent analyses

Preparation of plant extracts

The powdered sample (40g) was mixed with 200ml of n-Hexane (99.99%) and ethanol (95%$^v/_v$) respectively. Aqueous extract was prepared by mixing 40g of the powdered leaf samples with 200ml of cold distilled water (28±2°C) and hot distilled water (100°C), respectively. The mixtures were allowed to stand for 72h with constant stirring, and then filtered with a clean white muslin cloth. The ethanol and n-Hexane extracts were later air-dried at 28±2°C for 48h and reconstituted in 30% Dimethylsulphoxide (DMSO) by mixing 10g each extract with 50ml of 30% DMSO to make a concentration of 200mg/ml. The reconstituted ethanol and n-Hexane

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extracts were allowed to stand for 24h before sterilization was achieved using millipore membrane filter (0.22μ).

Collection and maintenance of test organisms

The clinical isolates of the selected enteric bacteria used for this study were obtained from the Federal Medical Center Owo, Obafemi Awolowo University Teaching Hospital Ile-Ife and Ekiti State University Teaching Hospital Ado-Ekiti, all in Nigeria, while the typed cultures were collected from Federal Institute of Industrial Research Oshodi (FIIRO) Lagos, Nigeria. The bacterial cultures were maintained in double strength Mueller-Hinton agar slant at three weeks interval.

Evaluation of antibacterial activity of plant extracts

All bacteria used were standardized to 0.5 McFarland standards ($6x10^8$ cfu/ml) according to Oyeleke *et al.* (2008). The antibacterial effect of the extracts was tested on the Clinical isolates and typed cultures of the species of *Serratia, Citrobacter, Enterobacter, Escherichia, Klebsiella, Proteus, Salmonella* and *Shigella* using agar diffusion method on Mueller-Hinton agar. Four holes were made on Mueller-Hinton agar plates using sterile 5mm diameter cork borer and equal volumes of the extracts were transferred into the holes using a sterile needle and syringe. The plates were allowed to stand for 15minutes for pre-diffusion of the extract to occur and were then incubated at $37^{O}C$ for 24h. Thereafter, the diameters of zones of inhibition were measured in millimeters. Dimethylsulphoxide and water were used as the blank controls, while a standard antibiotic disc containing 10mg of Ciprofloxacin (Maxicare, Nigeria) was used as positive control (Ogundare and Onifade, 2009).

Determination of the Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of plant extracts.

The minimum inhibitory concentration (MIC) of the extracts was determined using agar diffusion method (Leonard *et al.,* 2007). Six concentrations (25, 50, 75, 100, 150 and 200mg/ml) of the extracts were prepared. Molten Mueller-Hinton Agar plates were inoculated with standardized bacterial culture by radial streaking of the agar plates using sterile cotton swab. Four wells were made one each of the seeded agar plates using sterile 5mm diameter cork borer and equal volume different concentration of the extracts were poured into different well using sterile syringe and needle. Thereafter, the inoculated plates were incubated at $37^{o}C$ for 24h. The concentration of extracts with least zones of inhibition on the test organisms was recorded as minimum inhibitory concentration (MIC). While Minimum Bactericidal Concentration (MBC) for each of the extracts against susceptible organisms was determined by touching the zones of inhibition exhibited by different concentration, with a sterile loop and streak on Mueller-Hinton agar and incubated at $37^{o}C$ for 24h and observed for growth.

Fractionation of the crude extracts using column chromatography

The crude ethanol extract (40g) and n-hexane extract (40g) were suspended in 200ml of distilled water and partitioned with 200ml of n-hexane, 200ml of dichloromethane and 200ml of ethyl-acetate. The organic fractions of each were concentrated to dryness using rotary evaporator (Searchtech Instruments RES 2-2), weighed and labeled. Each fraction was subjected to Thin Layer Chromatography (TLC).

The hexane fraction of the crude extract was dissolved in 2ml of methanol and adsorbed unto silica and eluted with a mixture of n-hexane and ethyl-acetate. Fractions collected were analyzed by TLC in ethyl-acetate: ethanol ($1:1^{v}/v$). The resulting spots on the TLC plates were detected using of H_2SO_4 spray reagents. Fractions having the same TLC patterns / Rf were bulked together, concentrated *in vacuo* to dryness and weighed. Three fractions each were obtained for the ethanol extracts and n-hexane extracts and they were coded A1, A2 and A3 for ethanol extract fractions and B1, B2 and B3 for n-hexane extract fractions.

Antibacterial assay of crude fractions

Column chromatography was used to obtain different fractions of the chemical components (Bimal *et al.*, 2003).Disc diffusion method was used for this process. A 0.2g portion of each fraction was reconstituted with 4ml of 30%DMSO to make 50mg/ml each aseptically, Whattman No1 filter paper was cut into disc with the use of an office perforator, the discs were then sterilized in oven at $170^{o}C$ for 2h, and they were soaked in prepared fractions for 6 hours before used. The discs were aseptically placed on the agar plate surface seeded with the test organisms (bacteria) with sterilized forceps. The antimicrobials agents were allowed to diffuse from the disc to an inoculated agar plate for 1h and then incubated at $37^{o}C$ for 24h. The inhibition zones was observed and measured on the agar surface in places where the spots of antimicrobials discs were stacked to the agar (Das *et al.,* 2005 and Silva *et al.*, 2005).

Determination of the functional groups of the active compounds

A 0.1g portion of the fractionated sample was grinded with Potassium bromide (KBr) and compressed to form a transparent disc, the disc was then scanned in a SHIMADZU IR machine. The spectrum was printed out with the aid of the machine printer and the spectrum of each fraction was compared with those of some named compounds in the process of identifying the functional groups of the bioactive components of the plant extracts. The Infrared Resonance was done at the Chemistry Laboratory of the Redeemer's University of Nigeria (RUN), Ede. Osun State.

Phytochemical screening

The qualitative and quantitative analyses of the following phytochemicals in the test plant were carried out as described by Trease and Evans (1989): Saponins, Tannins, Flavonoids, Alkaloids, Phenols, Glycosides, Terpenes and Sterols, Cardenolides), Phlobatannins and Anthraquinones.

Statistical Analysis of Data

Data obtained from the study were subjected to one way analysis of variance (ANOVA) with five replicates

and treatment means were separated using Least Significant Difference (LSD) at 95% confidence intervals using SPSS window 7 Version 16.

Results

The results for the yield of the plant extracts in different extraction solvents as shown in Table 1 indicated that the same quantity (400g each) of the plant materials was used for the extraction in different solvent. W1 is the quantity of the powdered leave used for the crude extraction (400g each), W2 is the amount of the dried extract obtained after soaking for 72h with ethanol extract have the highest yield of 18.25% and cold water extract have the least yield with 11.50%. W3 is the amount of the dried extract taken for phytochemical screening (40ml each of the aqueous extract, 35g and 30g each of ethanol and n-hexane extract respectively). W4 is the weight of the fractions obtained after column chromatography separation, A1, A2, and A3 are the code names for the ethanol fractions with A2 having the highest weight of1.1grams and A1 with the lowest value of 0.85grams. B1, B2 and B3 are the code names for fractions (Table 1) obtained from the n-hexane extract with B3 having the highest value of 1.3grams while B2 has the lowest of 0.76grams. The results of antibacterial screening of the different extracts against the test isolates are shown in Tables 2. *Proteus vulgaris, Serratia marcescens, Klebsiella pneumoniae, Citrobacter freundii, Proteus mirabilis* ATCC 25933, *Serratia marcescens* ATCC 39006, *Klebsiella pneumoniae* ATCC 13883, *Citrobacter freundii* and *Shigella flexneri* ATCC 12022are all susceptible to ethanol extracts at 200mg/ml with *Proteus mirabilis* ATCC 25933 having the largest zones of inhibition of 10.8mm while *Proteus vulgaris* having the lowest zones of inhibition of 4.6mm, while *Escherichia coli, Salmonella typhi, Shigella flexneri, Escherichia coli* ATCC25922, *Salmonella typhi* ATCC 6539 *Citrobacter freundii* ATCC 8090and *Enterobacter aerogenes* ATCC 13048 are resistant to the ethanol extract. *Serratia marcescens* and *Klebsiella pneumoniae* are the only bacteria which were susceptible to 200mg/ml of n-hexane extracts of the leaves. Water extracts had no effects on any of the organisms tested, irrespective of the extraction temperature and extract concentration. The minimum inhibitory concentrations of the extract on the test isolates are shown in Table 3. The lowest minimum inhibitory concentration (MIC) of 75mg/ml was observed on *Shigella flexneri* ATCC12022, while the higher MIC of 150mg/ml ethanol extract was in *Proteus vulgaris and Klebsiella pneumoniae*. The lowest MIC of 100mg/ml was observed on both *Serratia marcescens* and *Klebsiella pneumoniae* for n-hexane extract. The minimum bactericidal concentrations (MBC) were also determined but the two extracts (ethanol extract and n-hexane extract) to which the organisms were susceptible did not exhibit minimum bactericidal concentrations (Table 4).

Table 1: Yield (g) of plant extracts in different extraction solvents

Extracts	W1 W1	W2 W2	W3W3/volume	W4					
				A1	A2	A3	B1	B2	B3
CWE	400.00	46.00 (11.50%)	40.00ml	--- --	-- --	-- --	-- --	-- --	-- --
HWE	400.00	48.00(12.00%)	40.00ml	-- --	-- --	-- --	-- --	-- --	-- --
EE	400.00	73.00 (18.25%)	35 (47.94%)	0.85	1.10	0 .97	-- --	-- --	-- --
n-HE	400.00	68.40 (17.10%)	30 (43.85%)	--	--	--	0.93	0.76	1.30

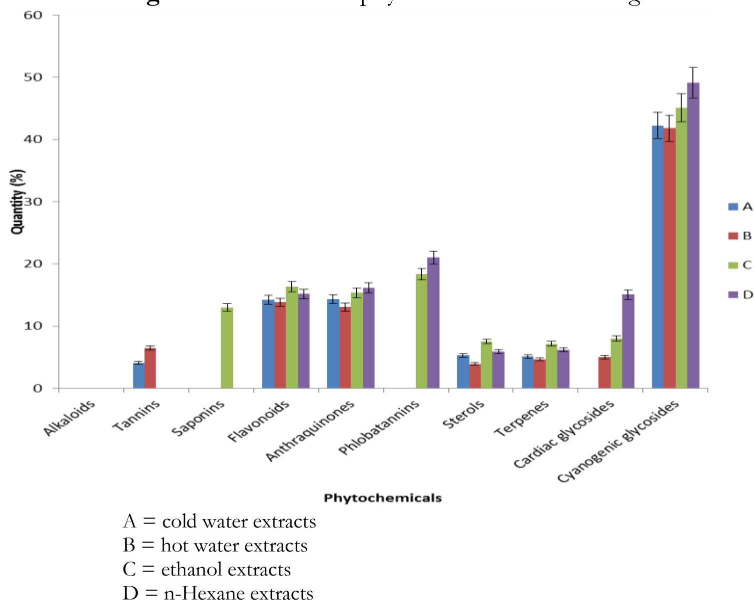
-- = Not determined CWE = Cold water extract HWE = Hot water extract
EE = Ethanol extract n-HE = n-Hexane extract
W1= Weight of powdered leaves soaked for crude extraction
W2 = Weight of extract obtained after air-drying.
W3 = Weight of sample taken for phytochemical analysis and column chromatography.
W4 = Weight of fractionated samples obtained
A1, A2, A3, B1, B2, and B3 = Code names of the fractions.

Table 2: Antibacterial effects of *R. madagascariensis* extract (200mg/ml) against clinical isolates and typed cultures of the test organisms.

Test organisms	ciproflo xacin	30% DMSO	Zones of inhibition (mm)			
			Ethanol extract	n-Hexane extract	Hot water extract	Cold water extract
Escherichia coli	23.00	-	-	-	-	-
Proteus vulgaris	25.00	-	4.60±0.01 (18)	-	-	-
Serratia marcescens	23.00	-	7.60±0.11 (33)	5.40±0.02 (24)	-	-
Klebsiella pneumoniae	20.00	-	5.60±0.03 (27)	4.80±0.04 (24)	-	-
Citrobacter freundii	20.00	-	6.00±0.01 (30)	-	-	-
Salmonella typhi	22.00	-	-	-	-	-
Shigella flexneri	20.00	-	-	-	-	-
Escherichia coli (ATCC25922)	20.00	-	-	-	-	-
Proteus mirabilis (ATCC 25933)	22.00	-	10.80±0.04 (46)	-	-	-
Serratia marcescens (ATCC 39006)	23.00	-	-	-	-	-
Klebsiella pneumoniae (ATCC 13883)	30.00	-	7.40±0.02 (25)	-	-	-
Citrobacter freundii (ATCC8090)	21.00	-	-	-	-	-
Salmonella typhi(ATCC 6539)	18.00	-	-	-	-	-
Shigella flexneri (ATCC 12022)	20.00	-	8.20 ± 0.01(41)	-	-	-
Enterobacter aerogenes(ATCC 13048)	16.00	-	-	-	-	-

Values are means of five replicates ± Standard error, Percentage inhibition over control in parenthesis.
- = No inhibition.

Figure 1: Amounts of phytochemicals in *R. madagascariensis*



A = cold water extracts
B = hot water extracts
C = ethanol extracts
D = n-Hexane extracts

Table 3: Minimum inhibitory concentration (MIC) of the extracts on susceptible clinical isolates and typed cultures

Microorganisms	Minimum inhibitory concentrations (mg/ml)	
	Ethanol extract	**n-Hexane extract**
Citrobacter freundii	100	-
Proteus vulgaris	150	-
Serratia marcescens	100	100
Klebsiella pneumoniae	150	100
Proteus mirabilis (ATCC 25922)	100	-
Kleb. pneumoniae (ATCC 13883)	100	-
Shigella flexneri (ATCC 12022)	75	-

Values are means of five replicates

Table 4: Minimum bactericidal concentration (MBC) of the extracts on susceptible clinical isolates and typed cultures

Microorganisms	Minimum bactericidal concentrations (MBC) (mg/ml)						
	Ethanol extract				**n-Hexane extract**		
	200	150	100	75	200	150	100
Citrobacter freundii	X	X	X				
Proteus vulgaris	X	X					
Serratia marcescens	X	X	X		X	X	X
Klebsiella pneumoniae	X	X			X	X	X
Proteus mirabilis(ATCC 25922)	X	X	X				
Klebsiella pneumoniae(ATCC 13883)	X	X	X				
Shigella flexneri (ATCC 12022)	X	X	X	X			

X = No inhibition

Table 5 shows the result of the phytochemical analysis obtained from each of the four extracts, only the ethanol extract contains saponins, while tannins is present only in the hot water and cold water extracts, all the extract contains hydroxyl anthraquinones, combined anthraquinones, cardenolides, lactone ring cardenolides, Cyanogenic glycosides, Terpenes, sterols flavonoids but only n-hexane and ethanol extract contained Phlobatannins. The quantitative analysis of the phytochemical compounds present shows that all the extracts contained 40-49% of Cyanogenic glycosides, about 10% of terpenes, 5-10% of sterols, 10-20% of anthraquinones and 12-20% of flavonoids. 13% saponin is only present in ethanol extracts, 4% and 6.5% of tannins is only present in cold and hot water extract respectively, about 20% of Phlobatannins is contained in the ethanol and n-hexane extracts, only the cold water extract contained no cardiac glycoside which is present in all the three remaining extracts and ranges between 5% and 15% (Figure 1).

Table 5: The phytochemical constituents of leaf extracts of *R. madagascariensis*

Phytochemicals	Extracts			
	A	**B**	**C**	**D**
Alkaloids	-	-	-	-
Saponins	-	-	+	-
Tannins	+	+	-	-
Phlobatannins	-	-	+	+
Anthraquinones (Borntragers test)	-	-	-	-
Hydroxyl-anthraquinones	++	++	++	++
Combined-anthraquinones	++	++	++	++
Cardiac glycosides test				
Legal test – cardenolides	+	+	+	+
Keddes test	+	+	+	+
(for the presence of lactones' ring in the cardenolides)				
Lieberman's test				
Cyanogenic glycosides	++	++	++	++
Terpenes and sterols	++	++	++	++
Flavonoids	++	++	++	++

\- = Not detected
\+ = Detected
++ = Detected in substantial amount
A = Cold water extract
B = Hot water extract
C = Ethanol extract
D = n-Hexane extract

The average zones of inhibition of the fractionated samples against the bacteria that were susceptible to the crude extract, A1 have the highest zone of inhibition against *Citrobacter freundii* with 23.00mm while the least was recorded in *Proteus mirabilis* (ATCC 25933) with 9.00mm. Sample B1 was only active against *Klebsiella pneumoniae* ATCC13883 and *Serratia marcescens* having the zone of inhibition of 4.00mm and 10.00mm respectively as presented in Table 6.

Table 6: Average zones of inhibition (mm) induced by different fractions of the plant extracts (50mg/ml) against the test organisms.

Organisms	Ciprofloxacin (20mg/ml)	Fractionated samples					
		Ethanol extracts	**n-Hexane extracts**				
		A1	**A2**	**A3**	**B1**	**B2**	**B3**
Proteus mirabilis ATCC 25933	22.00	9.00±0.02 (41%)	-	-	-	-	-
Serratia marcescens	23.00	21.00±0.03 (91%)	-	-	10.00±0.02 (43%)	-	-
Kleb. pneumoniae ATCC13883	30.00	12.00±0.02 (40%)	-	-	4.00±0.01 (13%)	-	-
Shigella flexneri 12022	20.00	15.00±0.02 (75%)	-	-	-	-	-
Citrobacter freundii	25.00	23.00±0.03 (92%)	-	-	-	-	-
Proteus vulgaris	25.00	18.00±0.03 (72%)	-	-	-	-	-
Klebsiella pneumoniae	20.00	16.00±0.02 (53%)	-	-	-	-	-

Values are means of five replicates ± Standard Error
Percentage inhibition over control in parenthesis.
\- = No inhibition

Discussion

From the present study, the results obtained indicated that ethanol extract of leaves of traveler's trees inhibited the growth of the clinical isolates; *Proteus vulgaris, Serratia marcescens, Klebsiella pneumoniae* and *Citrobacter freundii* and the following typed cultures; *Shigella flexneri* ATCC 12022*, Proteus mirabilis* ATCC 25933*, Serratia marcescens* ATCC 39006and *Klebsiella pneumoniae* ATCC 13883. While *Escherichia coli, Salmonella typhi, Shigella flexneri, Escherichia coli* ATCC 25922*, Salmonella typhi* ATCC 6539and *Enterobacter aerogenes* ATCC 13048 are resistant to ethanol extract. *Serratia marcescens* and *Klebsiella pneumoniae* are the only organisms that were susceptible to 200mg/ml of n-hexane extracts of the control plant with average zones of inhibitions of 5.40mm and 4.80 respectively, while all other bacteria tested were resistant to it. *Proteus mirabilis ATCC 25933* was the most susceptible bacteria with the zone of inhibition of 10.00mm while *Proteus vulgaris* showed the lowest zone of inhibition of 4.60mm against the crude ethanol extract. This therefore shows that the extract contains substance(s) that inhibits the growth of some microorganisms. Other works have also shown that extracts of plants inhibit the growth of some enteric bacteria (Sowmayanath, 2008). All microorganisms tested were resistant to aqueous extracts of the plant leaves, this means that water has no ability to extract the antibacterial compound(s) present in some plant leaves and this reason may have applied to the leaves of this plant, as this is in agreement with the work of Sowmayanath, (2008).

The non-susceptibility of *Escherichia coli, Salmonella typhi, Shigella flexneri, Escherichia coli ATCC 25922, Salmonella typhi ATCC 6539* and *Enterobacter aerogenes ATCC 13048* to the extracts may be due to the possession of a mechanism for detoxifying the active principles in the extract. Some bacteria are known to possess mechanisms by which they convert substances that inhibit their growth to non-toxic compounds. For examples *Escherichia coli, Salmonella typhi* and some other Enterobacteriaceae produces the enzyme penicillinase (β-lactamases) which converts the antibiotic penicillin to penicillinic acid which is no longer inhibitory to its growth (Prescott *et al.,*2005).

This may also be due to the pre-exposure of bacteria to similar antimicrobial compound resulting from drug abuse (in the case of the clinical isolates) or the continuous subculturing and transfer of the typed cultures causing environmental stress resulting to the development of gene(s) that conferred resistance of bacteria to the antimicrobial compounds (Singleton, 1999). The ability of the ethanol extract and n-hexane extract to cause the inhibitory effect but no killing effect on the test organisms may be due to the presence of bacteriostatic antimicrobial compounds in the extract (Sowmayanath, (2008).

The antibacterial effects of ethanol and n-hexane extracts may be due to the presence of flavonoids, anthraquinones and corroborative effect of Phlobatannins in the ethanol and n-hexane extracts. The observed antibacterial effects on the isolates may be due to the presence of alkaloids, tannins and flavonoids which have been shown to possess antibacterial properties (Draughon, 2004; Fadipe and Onifade, 2015). Some workers have also attributed the observed antimicrobial effects of plant extracts to the presence of these secondary metabolites (Sowmayanath, 2008). Some workers have also identified the presence of Phlobatannins and flavonoids in the extracts of the plant (Akujobi *et al*., 2004; Yoshida *et al*., 1990). The observed antibacterial properties corroborate the use of the plant in traditional medicine. Traditionally extracts of the plant are used in treating diarrhea (Sowmayanath, 2008).

In conclusion, temperature had no effect in the extraction of the active components of the leaves of Ravenala madagascariensis and water (cold and hot) has no ability to extract the bio-active compounds from the leaves of Ravenala madagascariensis. Ethanol and n-Hexane extracts of the leaves of Ravenala madagascariensis have proved to be effective in inhibiting the growth (i.e. showed a bacteriostatic but not bactericidal at 200mg/ml) of enteric bacteria such as *Shigella flexneri* ATCC 12022*, Serratia marcescens* ATCC 39006*, Klebsiella pneumoniae* ATCC 13883*, Proteus mirabilis* ATCC 25933*, Serratia marcescens, Klebsiella pneumoniae, Proteus vulgaris and Citrobacter freundii* which are among the causative agents of diarrhea.

The infrared spectrum of the extract showed that the extract contained functional groups such as hydroxyl (OH), carbonyl (C=O) of carboxylic acids, the aromatic ring and the C-O of the phenolic and alcohol group which are suggested to be components of anthraquinones and flavonoids that are present and which is the chief anti-diarrhea components of the extract. Therefore, it is recommended that further research on determination and removal of toxic components of this plant extracts should be done to ensure save recommendation to human for the treatment of diarrhea.

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