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Original Research Article

Phytochemical and antimicrobial screening of *Spondias mombin*, *Senna occidentalis* and *Musa sapientum* against *Vibrio cholerae* O1

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A B S T R A C T

Vibrio cholerae O1 can cause large epidemic of cholera with high mortality. This study investigated the phytochemical and antimicrobial properties of extracts of leaves of *Spondias mombin* and *Senna occidentalis* and stem sap of *Musa sapientum* against two epidemic strains of *V. cholerae* O1 (BA O1 and CVC O1). Aqueous and ethanolic extracts of *Spondias mombin* and *Senna occidentalis* were obtained using soxhlet extraction while the stem sap of *M. sapientum* was obtained fresh. The filtrates were dried at 40^{o}C and stored at 4^{o}C. The crude extracts were subjected to phytochemical analysis using standard methods. *In vitro* antimicrobial studies were investigated using microbroth dilution method to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Phytochemical analysis revealed the presence of tannins, saponins, alkaloids, anthraquinones, flavonoids, cardiac glycosides, phenols and phlobatannins. Aqueous and ethanolic leaf extract of *Spondias mombin* and aqueous leaf extract of *S. occidentalis* have vibriocidal activities with antimicrobial activity showing that *S. mombin* water extract (SMWE) had MIC (41.56mg/ml); and MBC (83.13mg/ml) against both BA O1 and CVC O1 respectively while the ethanolic extract (SMEE) had MIC (83.13mg/ml); and MBC (166.25mg/ml). *Senna occidentalis* water extract (SOWE) had MIC (166.25mg/ml); and MBC (332.50mg/ml) against BA O1 and CVC O1 respectively. Both aqueous and ethanolic extracts of *M. sapientum* and ethanolic extract of *S. occidentalis* were not effective *in vitro* on the epidemic strains tested. *Spondias mombin* and *Senna occidentalis* could be useful in drug research and development because of the phytochemicals that they possess.

K e y w o r d s

Phytochemical; Antimicrobial; *Spondias Mombin*, *Senna Occidentalis*; *Musa Sapientum*; Cholera; Nigerian Herbs.

Introduction

Cholera still poses a major treat every year causing large epidemic with high mortality with an estimated 3-5 million cases and 100,000 – 120,000 deaths worldwide (WHO, 2010). The causative agent, *Vibrio cholerae* is a gram-negative, motile, curved rod. It is divided into more than 70 serogroups defined by the O antigen, and

in which strains in O1 and O139 are associated with epidemic cholera. They are further characterized by biotype (El Tor biotype and Classical biotype), serotype (Inaba, Ogawa, and Hikojima) and the non-O1 serotype, and production of cholera toxins (CDC, 2011).

V. cholerae is transmitted through contaminated water and food. The source of contamination in epidemic is faeces of cholera patients, and usually consequences of poor sanitation. Marine environments may serve as long-term reservoirs. A faecal-oral infection is also possible. The infective dose of cholera is 10^6 viable cells per person, but it varied with age and health (CDC, 2011). The incubation period ranges from 1 to 5days, but is usually 2days. Symptoms are characterized by sudden onset of profuse watery diarrhoea, colloquially referred to as "rice-water stool" and vomiting, loss of fluid and electrolytes in dehydration. Painful muscle cramps, blue skin and clouded mental status may occur in more severe cases. An untreated cholera person may produce around 10 litre of diarrhoea fluid a day (Fakruddin *et al.,* 2011).

Case management of cholera requires assessment of hydration status, rehydration therapy, and antimicrobials therapy. The treatment of infectious disease is mainly based on the use of antibiotics. A number of antibiotics have lost their effectiveness due to development of resistant strains (Sivapriya *et al.,* 2011).

The rapid emergence of multiple antibiotic resistant strain of *V. cholerae O1* and inadequacy of drugs in disease treatments has called for the development of alternative therapeutic agents which are newer and effective. Spondia *mombin, Senna occidentalis and Musa sapientum* have been used locally in treating diarrhoea, dysentery and other gastrointestinal diseases but reports on their use against *V. cholerae O1* is very scanty (Shariff, 2001).

Spondia mombin is widely relied on various herbal remedies for numerous conditions and virtually every part of the tree is used from its thickly corky bark, to its leaves, fruits and even its flower. It has been reported that the plant extract has antibacterial (Abo *et al.,* 1999), antiviral (Corthout *et al.,* 1992), Antihelminthic (Ademola *et al.,* 2005) and molluscicidal (Corthout *et al.,* 1994) activities. The leaves have also been shown to exhibit abortifacient and lipid lowering actions (Igwe *et al.,* 2008). The reported use of the plant's leaf extract as an abortifacient or labour–inducing agent on one hand, and also for prevention of miscarriages on another are mutually exclusive sides of a coin. Tannins, saponins, flavonoids, sterols and quinine are chemicals found in the plant (Taylor, 2006).

Senna occidentalis L called as "*ewe ori esi"* in Yoruba and Coffee Senna in English belongs to family Caesalpiniaceae, subfamily Caesalpinioideae. It is an ayurvedic plant with huge medicinal importance (Arya *et al.,* 2010). Leaves of *S. occidentalis* plant have ethnomedicinal importance like paste of leaves is externally applied on healing wounds, sores, itch, cutaneous diseases, bone fracture, fever, ringworm, skin diseases and throat infection. Previous pharmacological investigations showed that *S. occidentalis* leaf extracts have antibacterial (Saganuwan and Gulumbe, 2006), antimalarial (Tona *et al.,* 1999), antimutagenic (Jafri *et al.,* 1999; Sharma *et al.,* 2000), antiplasmodial (Tona *et al.,* 2004), anticarcinogenic (Sharma *et al.,*

2000) and hepatoprotective (Yadav *et al.,* 2009) activity. Moreover, studies on this plant showed that the nature and amount of the phytochemicals varies according to the season and geographical location (Yadav *et al.,* 2009).

Musa sapientum (Latundan Banana) with Banana as the common name for the herbaceous plants of the genus *Musa* is very important for the fruit produce. Stem juice of fruited plant is used for the treatment of diarrhoea, dysentery, cholera, otalgia, haemoplysis (Ghani, 2003). Banana flakes has also been tested and found effective in the treatment for diarrhoea in critically-ill patients receiving enteral feeding (Emery, 1997), also in children (Rabbani *et al.,* 1999). Ethanolic and aqueous extracts have been said to have antimicrobial activities against *S. aureus, S. paratyphi, Shigella flexineri, E. coli, K. pneumonia, B. subtilis and P. aeruginosa*. Also, it has bacteriostatic activity against *B. cereus, B. coagulans, B. stearothermophilus, and Clostridium sporogenes* (Rabbani *et al.,* 1999).

This research was designed to evaluate the antimicrobial property of *Spondias mombin, Senna occidentalis* and *Musa sapientum*, which could justify their use as antimicrobial agent against epidemic strains of *Vibrio cholerae* O1.

Materials and Methods

Collection of Plant Samples

Leaves of *Spondias mombin, Senna occidentalis* and stem of *Musa sapientum* were harvested fresh from the Federal University of Agriculture, Abeokuta, Nigeria and identified at the herbarium of University of Ibadan, Nigeria.

Preparation of Plant Materials

Fresh leaves of *Spondias mombin, Senna occidentalis* and stem of *Musa sapientum* collected were washed under running tap water, air-dried at ambient temperature and then reduced to coarse powder using an electric blender. The powders obtained were stored in different airtight containers prior to extraction. The dried powder leaves and stems were successively extracted using Soxhlet apparatus involving different mix of solvents (40% aqueous-ethanol and water).

Soxhlet Extraction

Eighty grammes of each powdered sample was placed inside a thimble made from thick filter paper. The thimble was then placed onto a flask containing the extraction solvent and then equipped with a condenser. The solvent is heated to reflux at 80^{o}C. The solvent vapour travels up a distillation arm and floods into the thimble. The condenser ensures that any solvent vapour cools, and drips back down into the thimble. The chamber containing the plant sample slowly fills with warm solvent dissolving some of the desired compounds. When the thimble is almost filled up, it automatically empty itself by a siphon side arm, with the solvent running back down to the distillation flask. This cycle was allowed to repeat many times until a clear solvent was observed in the thimble. During each cycle, a portion of the non-volatile compound dissolves in the solvent.

Concentration of Extracts

The extracts in the distillation flask is then filtered using Whatman No. 1 Filter paper into a conical flask. The filtrates were concentrated by evaporating to dryness in a hot air oven at 40^{o}C and then stored at 4^{o}C.

Sample Reconstitution and Preparation for Use

For the preparation of dilutions of crude extracts for antimicrobial assay, the extracts were reconstituted by dissolving in the respective extracting solvent and diluted further for assay of antimicrobial activity.

Phytochemical Screening

The crude plant extract obtained was subjected to preliminary phytochemical screening, to identify the chemical constituents. The methods of analysis employed were those described by Harbone and Baxter (1993); Sofowora (1993).

Test for Flavonoids

Extract (0.5g) was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips was added to the filtrate followed by few drops of concentrated HCl. A pink orange or red to purple coloration was in evidence for the presence of flavonoids.

Test for Tannins

To the filtrate, 2ml of Ferric's reagent was added. A dark green colouration indicates the presence of tannins.

Test for Alkaloids

About 0.5g of aqueous extract stirred in 5ml 1% HCl was boiled in water bath and filtered. To the filtrate, few drops of Mayer's reagent were added. A creamy or white precipitate indicates the test positive.

Test for Saponins

Extract (1g) was boiled with 5ml distilled water, allowed to cool and then filtered. The filtrate was mixed with 3ml distilled water and then shaken vigorously for a stable persistent froth. Frothing which persist is an indication of the presence of saponins.

Test for Phlobatannins

Aqueous HCl (1%) was added to the extract. A red precipitate was an indication of the presence of phlobatannins.

Test for Anthraquinones

Extract (1g) was added to 10ml benzene, filtered and ammonia solution added. The presence of rose-pink colour in the aqueous layer indicates the presence of anthraquinones

Test for Phenolic Compound

The extract (300mg) was diluted with 5ml of distilled water and 5% Ferric chloride was added to the filtrate. A dark-green colour was an indication of the presence of phenolic compound.

Test for Cardiac Glycosides

Extract (0.5g) was added to 2ml acetic anhydrate plus concentrated H_2SO_4. A purple to reddish brown ring is an indication of the presence of cardiac glycosides.

Antimicrobial Studies

Source of *Vibrio cholerae* strains

Bauchi isolate (BA O1) of *Vibrio cholerae* serogroup O1, biotype El tor, serotype Ogawa and controlled strain of *Vibrio cholerae* (CVC O1) serogroups O1, biotype El tor, serotype Inaba from Centre

of Disease Control (CDC) Kenya were obtained from the Department of Molecular Biology and Biotechnology, Nigerian Institute of Medical Research (NIMR), Yaba, Lagos State.

Preparation of Inoculum of *Vibrio cholerae*

A pure isolate of *V. cholerae* was inoculated on a Mueller Hilton agar and incubated at 37^{o}C for 24 hrs. A uniform suspension of the overnight pure culture of the *V. cholerae* was made with sterile normal saline. The concentration was then adjusted until it matches 0.5Mc Farland turbidity standard.

Sensitivity Testing *against Vibrio cholerae* strains

This was carried out by emulsifying a small inoculum of colonies of *V. cholerae* isolate grown overnight in 3ml sterile saline solution in a sterile bottle with 0.5 McFarland standard. Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the solution of bacterial culture and used to inoculate the Mueller-Hinton Agar. The inoculated plates were allowed to dry. A 0.1ml aliquot of the filtered extracts was introduced on the plates and incubated for 24h at 37^{o}C. The zones of inhibition were then measured in millimetres (mm) (Akinsinde and Olukoya, 1995).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Determination

The minimum inhibitory concentrations of the aqueous and ethanol extracts of each plant material were determined using broth microdilution methods. Each well of the microtitre plate was filled with 100µl of MH broth except the first well, which received 100µl of the herbs to be tested, with the extracts prepared at concentration of 1.3g/ml. This was then mixed together, and 100µl of the mixture was transferred to the next column of wells in a process of 1:2 dilutions until the eleventh well. The wells received an equal volume (20µl) of fixed bacterial culture and then incubated at 37^{o}C for 24hrs. The inoculum size for the microtitre plate procedure was 10^{6}cfu/ml. Therefore, the plates were examined for changes in turbidity as an indicator of growth.

The first well that appears clear was taken to be the MIC of the extract. The minimum bactericidal concentration was determined by subculturing the preparation that showed no growth in the MIC assay onto MH agar and further incubated at 37^{o}C for 24hrs. The lowest concentration that showed no growth on the MH agar was the MBC.

Results and Discussion

Organoleptic Properties Determination and its Extractive Value of Plant Extract in Different Solvents

The organoleptic properties of the plant extracts and their extractive values (Table 1) shows that *S. occidentalis* ethanol solvent gave the highest yield at 42.58% followed by *S. mombin* water solvent (40%), *S. occidentalis* water solvent (39.94%), *S. mombin* water solvent (33.33%), *M. sapientum* water solvent (26.67%) and *M. sapientum* ethanol solvent (25%). In response to the colour of the extract, water extract of *S. mombin* and ethanol extract of *S. mombin, S. occidentalis and M. sapientum* have a characteristic dark brown colour, while *S. occidentalis* and *M. sapientum* water

extract have a dark green and light brown colour respectively. Sticky mass texture were observed in water extract of *S. occidentalis* and ethanol extract of *S. mombin* and *S. occidentalis* while dried powder extract were observed in ethanol extracts of *M. sapientum* water extract of *S. mombin and M. sapientum.*

Phytochemical Screening of Aqueous and Ethanol extracts of *Spondias mombin, Senna occidentalis* and *Musa sapientum*

The phytochemical screening of aqueous and ethanol extract of *Spondias mombin, Senna occidentalis* and *Musa sapientum* (Table 2) showed that tannins and flavonoids were present in all the extract, while cardiac glycosides was absent only on SOWE. SOEE out of the remaining extract had phlobatannins. Phenols are present both in SMEE and SOWE while absent in the other extracts. Anthraquinones and saponins were present only in SMWE, SMEE and SOWE, while alkaloids were only present in SMWE, SMEE and SOWE respectively.

Susceptibility of *Vibrio cholerae strains* to the Crude Plant Extract

Table 3 shows the antimicrobial activity of the crude plant extract against epidemic strains of *V. cholerae* with SMWE having the highest activity, followed by SMWE and then SOWE with diameter of zone of inhibition against BA O1 (25mm, 22mm and 21mm) and CVC O1(24mm, 22.5mm and 21mm) respectively. However, SOWE, MSWE and MSEE showed no visible zone of inhibition against the epidemic strains of *V. cholerae*.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Active Plant Extracts Against BA O1and CVC O1

Table 4 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of active plant extracts against the epidemic strains of *V. cholerae*. The extracts (SMWE, SMEE and SOWE) shows similar activities with their MICs as 41.56mg/ml, 83.13 mg/ml and 166.25 mg/ml respectively and their MBC as 83.13 mg/ml, 166.25 mg/ml and 332.50mg/ml respectively for both epidemic strains..

Epidemic causing cholera bacteria are rapidly becoming resistant to conventional drugs (Karim *et al.,* 2009). In the past two decades, *V. cholerae* serotype O1 have been reported to be resistant to tetracycline, streptomycin, ciprofloxacin, erythromycin (Jain *et al.,* 2008) even in several endemic countries including India and Bangladesh.

In the continuation of new antimicrobial drug discovery, aqueous and ethanol extract of *Spondias mombin, Senna occidentalis and Musa sapientum* were investigated. Although these plants have been known to posses antibacterial properties and have been used to treat several diarrheal, dysentery and other gastrointestinal diseases however, reports on their use against *V. cholerae O1* is very scanty. Thus in assessment to evaluate the antimicrobial properties of these plants against epidemic strains of *V. cholerae*, this research was conducted.

Table.1 Organoleptic Properties Determination and its Extractive Value of Plant Extract in Different Solvents

Plants	Plant Part	Solvent Used for Extraction	Colour of the Extracts	Texture of the Extracts	Yield (%)
S. mombin	Leaves	Water	Dark brown	Dried powder	40.00
S. mombin	Leaves	Ethanol	Dark brown	Sticky mass	33.33
S. occidentalis	Leaves	Water	Dark green	Sticky mass	42.58
S. occidentalis	Leaves	Ethanol	Dark brown	Sticky mass	39.94
M. sapientum	Stem	Water	Light Brown	Dried powder	26.67
M. sapientum	Stem	Ethanol	Dark brown	Dried powder	25.00

Table.2 Phytochemical Screening of Aqueous and Ethanol extracts of *Spondias mombin*, *Senna occidental*is and *Musa sapientum*

PHYTOCHEMICALS	SMWE	SMEE	SOWE	SOEE	MSSE	MSEE
Tannins	+	+	+	+	+	+
Anthraquinones	+	+	+	-	-	-
Flavonoids	+	+	+	+	+	+
Cardiac Glycosides	+	+	-	+	+	+
Saponins	+	+	+	-	-	-
Alkaloids	+	+	-	+	-	-
Phenols	-	+	+	-	-	-
Phlobatannins	-	-	-	+	-	-

Activity Key: + = Present; - = Absent

Key: SMWE=*Spondias mombin* water extract; SMEE=*Spondias mombin* ethanol extract; SOWE=*Senna occidentalis* water extract; SOEE= *Senna occidentalis* ethanol extract; MSSE=*Musa sapientum* stem extract; MSEE=*Musa sapientum* ethanol extract

Table.3 Susceptibility of *Vibrio cholerae* strains to the Crude Plant Extract

Extracts	Isolates/Zone of Inhibition (mm)	
	BA O1	CVC O1
Spondias mombin Water Extract (SMWE)	25	24
Spondias mombin Ethanol Extract (SMEE)	22	22.5
Senna occidentalis Water Extract (SOWE)	21	21
Senna occidentalis Ethanol Extract (SOEE)	NZI	NZI
Musa sapientum Water Extract (MSWE)	NZI	NZI
Musa sapientum Ethanol Extract (MSEE)	NZI	NZI

Key: NZI = No zone of inhibition; BA O1= Bauchi isolate of *Vibrio cholerae* serogroups O1; CVC O1= Kenya isolate of *Vibrio cholerae* serogroups O1.

Table.4 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Active Plant Extracts Against BA O1and CVC **O1**

Organism	**BA O1**		**CVC O1**	
Extract	**MIC (mg/ml)**	**MBC (mg/ml)**	**MIC (mg/ml)**	**MBC (mg/ml)**
SMWE	41.56	83.13	41.56	83.13
SMEE	83.13	166.25	83.13	166.25
SOWE	166.25	332.50	166.25	332.50

Key: SMWE = *Spondias mombin* water Extract; SMEE = *Spondias mombin* Ethanol Extract; SOWE = *Senna occidentalis* water Extract; BA O1= Bauchi isolate of *Vibrio cholerae* serogroups O1; CVC O1= Kenya isolate of *Vibrio cholerae* serogroups O1.

Among all the plant extracts, water extract of *S. occidentalis* was found to have the highest extractive value of 42.5% followed by *S. mombin* water extract (40%), *S. occidentalis* ethanol extract (39.94%), *S. mombin* ethanol extract (33.33%), *M. sapientum* water extract (26.67%) and *M. sapientum* ethanol extract having the least yield of 25%. This result shows that water used as solvent in extraction process have more extractive power than ethanol.

The colour of the plant extracts in different solvent were characterised with water extract of *S. mombin* and ethanol extracts of *S. mombin*, *S. occidentalis* and *M. sapientum* have a characteristic dark brown colour, while *S. occidentalis* and *M. sapientum* water extract have a dark greens and light brown colour respectively. Sticky mass texture were observed in water extract of *S. occidentalis* and ethanol extract of *S. mombin* and *S. occidentalis* while dried powdered extract were observed in ethanol extract of *M. sapientum* and water extract of *S. mombin* and *M. sapientum*.

Phytochemical screening of aqueous and ethanol of the plant extract of the plant (*S. mombin, S. occidentalis,* and *M. sapientum*) helps to reveal the chemical nature of the constituents of the plant extract which may also be used to search for bioactive agents that could be used in the synthesis of very useful drugs (Sundaran, 2010). Phytochemical screening of the leaves and stem extracts of the selected plants reveals the presence of Tannins, Anthraquinones, Flavonoids, Cardiac glycosides, Saponins, Alkaloids, Phenols, and phlobatannins. It shows that extracts of these plants all contain tannins and flavonoids, while cardiac glycosides were only absent in *S. occidentalis* water extract, *S. occidentalis* ethanol extract out of the remaining extract had phlobatannins. Phenol are present both in S. mombin ethanol extract and *S. occidentalis* water extract while absent in other extracts. Anthraquinones and saponins were present only in *S. mombin* water extract, *S. mombin* ethanol extract, and *S. occidentalis* water extract, while alkaloids were only present in *S. mombin* water extract, *S. mombin* ethanol extract, and *S. occidentalis* ethanol extract respectively.

Result of phytochemical content of aqueous and ethanol extract of *Senna occidentalis* was not in agreement with the work done by Ogunkunle and Ladejobi (2006) but in agreement with the work of Nuhu and Aliyu (2008), which could be attributed to the geographical locations of the plant. Phytochemical exert antimicrobial activity through different

mechanisms; tannins for example, act by iron deprivation, hydrogen binding or specific interactions with vital proteins such as enzymes in microbial cells (Scalbert, 1991; Akinpelu *et al.,* 2008). Tannins are well known for their anti-oxidant and anti-microbial properties, as well as for soothing relief, skin regeneration, as anti-inflammatory and diuretics (Okwu and Okwu, 2004). Herbs that have tannins as their component are astringent in nature and are used for the treatment of gastrointestinal disorders such as diarrhoea and dysentery (Dharnananda, 2003). This may therefore explain the use of *S. mombin, S. occidentalis* and *M. sapientum* in folklore remedy for gastrointestinal ailments and typhoid (Anthonia and Olumide, 2010).

Saponins, which is responsible for numerous pharmacological properties (Estrada *et al.,* 2000) was also tested positive in one or more of the plant materials examined. Saponins are considered a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effect (Liu and Henkel, 2002). They lower the cholesterol level; have anti-diabetic and anti-carcinogenic properties (Trease and Evans, 1989). In addition, Saponins are expectorants, cough suppressants and for haemolytic activities (Sofowora, 1993; Okwu, 2005). Alkaloids, the most revered of all the phytochemicals, are said to be pharmacologically active and their actions are felt in the autonomic nervous system, blood vessels, promotion of diuresis, respiratory system, gastrointestinal tract, uterus, malignant diseases, infections and malaria (Trease and Evans, 1989). In addition, alkaloids are antispasmodiac, analgesic and also have bactericidal effects (Okwu and Okwu, 2004). Alkaloids is present in some of the plant material has a common biological properties which is their toxicity against cells of foreign organisms (Akinpelu *et al.,* 2008). Also, flavonoids are reported to be important antimicrobial component (Chung *et al.,* 1998; Karou *et al.,* 2005). They are powerful antioxidant polyphenolic compounds (Nuhu and Aliyu, 2008). Torell *et al.* (1986) and Faure *et al.* (1990) have shown that flavonoids inhibit peroxidation of polyunsaturated fatty acids in cell membranes. Moreover, results have shown that flavonoids from *Helichrysum* genus inhibit the formation of superoxide ions and hydroxyl radicals, which are two strong peroxidation agents (Facino *et al.,* 1990) There are also enough documented dates, which suggest that there is a positive correlation between total phenolic content and antimicrobial activity (Shan *et al.,* 2007; Wu *et al.,* 2006; Kudo *et al.,* 2004). Cardiac glycoside acts on the heart muscles and increase renal flow (diuresis), while phlobatannins on the other hand, have astringent or styptic properties (Omotayo and Borokini, 2012).

The evaluation of herbs tested for vibriocidal activity against the epidemic strains of *V. cholerae* showed that some of the antidiarrhea herbs tested namely *S. mombin, S. occidentalis* and *M. sapientum* exhibited high activity while some did not exhibit any activity. This was shown using standard method. None of these medicinal plants were among those used by Akinsinde and Olukoya (2005) which are *Entada africana, Fiscus capensis, Lannea acida, Mimosa pucida, Mitragyna stipulosa, Piliostigma recticuatum and Terminalia avicennoides* in which *Terminalia avicennoides* showed the most active antimicrobial activity against *V. cholerae*. Also, *Acalypha indica* (Jayakumari *et al.,* 2010) and *Solanum torum* (Sivapriya *et al.,* 2011) and

Terminalia arjuna (Fakruddin *et al.*, 2011) have been tested against multi antibiotic resistant *V. cholerae*.

Of the three plant used, result showed that both aqueous extract of *S. occidentalis* (SOWE) and both aqueous and ethanol extract (SMWE, SMEE) had antimicrobial activity while ethanol extract of *S. occidentalis* (SOEE) and both aqueous and ethanol extracts of *M. sapientum* showed no activity against the epidemic stains (MSSE, MSEE). *S. mombin* water extract exhibited the highest zone of inhibition against BA O1 of 25mm, followed by *S. mombin* ethanol extract and *S. occidentalis* water extract with 22mm and 21mm respectively. So also activity against CVC O1 with *S. mombin* water extract (24mm), *S. mombin* ethanol extract (22.5mm) and *S. occidentalis* water extract (21mm). This result relates to the work done by Alma *et al.* (2003), also resistance of *M. sapientum* extracts was in consonance with previous work done by Valarmathy *et al.* (2010).

The minimum inhibitory concentration (MIC) assay was carried out using microbroth dilution on Mueller-Hinton broth for more accurate and precise methodology for productivity, repeatability and data integrity (Kobayashi *et al.,* 2004; Bradshaw, 2000). All the active extracts showed similar activity against both strains. The extracts (*S. mombin* water extract, *S. mombin* ethanol extract and *S. occidentalis* water extract) showed similar values with their MICs as 83.13mg/ml, 166.25 mg/ml and 332.50 mg/ml respectively and their MBCs as 41.56 mg/ml, 83.13 mg/ml and 166.25 mg/ml respectively for both epidemic strains. *S. mombin* water extract had the highest inhibitory and bactericidal concentration against the strains with MIC and MBC (83.12 mg/ml and 41.56 mg/ml), followed by *S. mombin* ethanol extract (166.25 mg/ml and 83.13 mg/ml) and *S. occidentalis* water extract having the least activity (332.5 mg/ml and 166.25 mg/ml). It is worthy of note that MBC values obtained for the extract against the pathogen are higher than MIC, indicating the extracts are bacteriostatic at lower concentration and bactericidal at higher concentration (Akinyemi *et al.,* 2006). The very potent activity of *S. mombin* extract against the epidemic strains of *V. cholerae* conforms to the study, which indicated that *S. mombin* leaves had beta-lactamase, an enzyme produced by certain bacteria that inactivates penicillin and results in response of antibiotics (Coates *et al.*, 1994).

This study thus confirms the traditional claims on these plants as a remedy to diarrhoea and dysentery. This is similar to conclusion drawn by Ajayi and Akintola (2010) after examining the antibacterial activity of some medicinal plants on common enteric food-borne pathogens and Abo *et al.* (1999), after evaluating the antimicrobial potential of *Spondias mombin, Croton zambesicus* and *Zygotritonia crocea*. This study thus validates the use of aqueous and ethanol extracts of *Spondias mombin* and aqueous extract of *Senna occidentalis* in diarrhoea and cholera treatment.

Contribution to Knowledge

Phytochemical constituents present in plant varies with the solvent used for extraction.

Both ethanolic and aqueous extracts of *Spondias mombin* and aqueous extract of *Senna occidentalis* possess *in vitro* vibriocidal activity while the ethanolic extract of *Senna occidentalis* and extracts

of *Musa sapientum* did not show vibriocidal activity.

Herbs are an integral part of nature. Plants contain natural substance that can promote health. From the present investigations it can be concluded that the vibriocidal properties of these plants; *Spondias mombin* and *Senna occidentalis* may be due to the combined or individual effect of the phytoconstituents found, which can be further confirmed by the extensive studies. The antimicrobial activities of these plants highlighted the importance of the extracts in traditional preparations. Based on the above results we can further conclude that the *Spondias mombin* and *Senna occidentalis* plants may be helpful in treating epidemic strains of *Vibrio cholerae* diseases in future days.

References

Abo, K.A., Ogunleye, V.O. and Ashidi, J.S. 1999. Antimicrobial potential of Spondias mombin, Croton zambesicus and Zygotritonia crocea. Phytotherapy Research 13: 494-497.

Ademola, I.O., Fagbemi, B.O. and Idowu, S.O. (2005). Antihelminthic Activity of extracts of Spondias mombin against gastrointestinal nematodes of Sheep: Studies *in vitro* and *in vivo*. Tropical Animal Health Production 37: 223-225.

Ajayi, A.O. and Akintola, T.A. 2010. Evaluation of antibacterial activity of some medicinal plants on common enteric food-borne pathogens. African Journal of Microbiology Research 4(4): 314-316.

Akinpelu, D.A., Adegboye, M.F., Adeloye, O.A. and Okoh, A.I. 2008. Biocidal activities of partially purified fractions from methanolic extract of Garcinia Kola (Heckel) seeds on bacterial isolates. Biology Research 41: 277-287.

Akinsinde, K.A. and Olukoya, D.A. 1995. Vibriocidal activities of some local herbs. Journal of Diarrhoeal Disease Research 13(2): 1-3.

Akinyemi, K.O., Oluwa, O.K. and Omomigbehin, E.O. 2006. Antimicrobial activity of crude extracts of three medicinal plants used in south-west Nigerian folk medicine on some food borne bacterial pathogens. African Journal of Traditional, Complementary and Alternative Medicines 3(4): 13-22.

Alma, M.H., Mavi, A., Yildirim, A., Digrak, M. and Hirata, T. 2003. Screening chemical composition and in vitro antioxidant and antimicrobial activities of the essential oils from Origanum syriacum L. growing in Turkey. Biological and Pharmaceutical Bulletin 26:1725-1729.

Anthonia, O. and Olumide, O. 2010. *In vitro* antibacterial potential and synergistic effect of South Western Nigerian plant parts used in folklore remedy for Salmonella typhi Infection. Nature and Science 8(9): 52-59.

Arya, V., Yadav, S., Kumar, S. and Yadav, J.P. 2010. Antimicrobial activities of Cassia occidentalis l. (leaf) against various human pathogenic microbes. Life Sciences and Medicine Research 9: 1-11.

Bradshaw, J.T. 2000. Determining dilution accuracy in microtiter plate assay using the Artel Multichannel Verification System (MVS). Artel. pp. 1-29.

Centre for Disease Control 2011. Haiti cholera training manual: a full course for healthcare providers. Pp. 1-48.

Chung, K.T., Wong, T.Y., Wei, C.I., Haung, Y.W. and Lin, Y. 1998. Tannins and human health: a review. Critical Review of Food Science Nutrition 38:421-464.

Coates, N.J., Gilpin, M.L., Guynn, M.N., Lewis, D.E., Milner, P.H., Spear, S.R. and Tyler, J.W. 1994. A novel beta-lactamase inhibitor isolated from Spondias mombin. Journal of Natural Products 57:654-657.

Corthout, J., Pieters, L.A., Claeys, M., Geerts, S.T., Vanden-berghe, D. and Vlietinck, A.J. 1994. Antibacterial and molluscicidal phenolic acids from Spondias mombin. Plants and Medicine 60: 460-463.

Corthout, J., Pieters, L.A., Claeys, M., Vanden-berghe, D.A. and Vlietinck, A.J. 1992. Antiviral caffeoyl esters from Spondias mombin. Phytochemistry 31(6): 1979-2019.

Dharnananda, S. G. 2003. The uses of tannins in Chinese medicine. *In Proceedings of Institute for Traditional Medicine* Portland, Oregon.

Emery, E.A. 1997. Banana flakes control diarrhea in enterally fed patient. Nutrition and Clinical Practice 12(2): 72-75.

Estrada, A., Katselis, G.S., Laarveid, B. and Bari, B. (2002). Isolation and evaluation of immunological adjuvant activities of Saponins from Polygaja senega L. comparative immunology. Microbial Infectious Diseases 23: 27-43.

Facino, M.R., Carini, M., Franzoi, L. and Pirola, O. 1990. Phytochemical characterization and radical scavenger activity of flavonoids from Helichrysum italicum G. Don (Compositae). Pharmacology Research 22: 709-721.

Fakruddin, M., Alam, K.M.A., Mazumdar, R.M., Islam, S., Nipa, M.N., Iqbal, A. and Bhuiyan, H.R. 2011. Antibacterial activity of the extract of terminalia arjuma against multi antibiotic resistant *Vibrio cholerae*. Journal of Scientific Research 3(1): 129-137.

Faure, P., Roussel, M.A. and Richard, M.J. 1990. Effect of acute zinc depletion on rat lipoprotein distribution and peroxidation. Biological Trace Element Research; 28:134- 146.

Ghani, A. 2003. Medicinal plants of bangladesh with chemical constituents and uses. Asiatic Society Bangladesh 2: 402.

Harbone, J.B. and Baxter, H.H. 1993. Phytochemical Dictionary. *A Handbook of Bioactive Compound from Plants.* Taylor and Francis, Washington, D.C., U.S.A. pp 237.

Igwe, C.U., Ojiako, O.A., Nwaogu, L.A. and Onyeze, G.O.C. 2008. Lipid lowering effects of aqueous leaf extract of Spondias mombin Linn. The International Journal of Pharmacology 6(1): 1-9.

Jain. M., Kumar, P. and Goel, A. 2008. Class 1 integrons and SXT elements conferring multidrug resistance in Vibrio cholerae O1 strains associated with a recent large cholera outbreak in Orrissa, Eastern India. International Journal of Antimicrobial Agents 32: 459-460.

Jayakumari, M., Maheswari, K., Subashree, M., Umamaheswari, M., Mala, P., Sevanthi, I. and Lakshmi, S. 2010. Antibacterial potential of *Acalypha indica* against human pathogens. International Journal of Current Research Vol. 1 , pp. 001 - 004.

Karim, M.R., Khandoker, A.K.M., Habib, M.R., Nikkon, F. and Rahman, M. 2009. Antibacterial and

antineoplastic effect of root of Euphorbia hirta l. Drug Invention Today 1(1): 10-12.

Karou, D., Dieko, H.M., Simpore, J. and Traore, S.A. 2005. Antioxidant and antibacterial activities of polyphenols from ethnomedical plants from Burkina Faso. African Journal of Biotechnology 4:823-828.

Kobayashi, I., Muraoka, H., Saika, T., Nishida, M., Fujioka, T. and Nasu, M. 2004. Micro-broth dilution method with air-dried microplate for determining MIC's of clarithomucin and amoxicillin for Helicobacter pylori Isolates. Journal of Medical Microbiology 53: 403-406.

Kudo, Y.H., Kobayashi, A., Konishi, Y.S. and Kondo, K. 2004. Antibacterial activity of plants used in cooking aroma and taste. Journal of Food Protocol 67:2820-2824.

Liu, J. and Henkel, T. 2002. Traditional Chinese medicine (TCM): are polyphenols and saponins the key ingredients triggering biological activities? Current Medical Chemistry 9: 1483-1483.

Nuhu, A.A. and Aliyu, R. 2008. Effects of Cassia occidentalis aqueous leaf extract on biochemical markers of tissue damage in rats. *Tropical* Journal of Pharmaceutical Research 7(4): 1137-1142.

Ogunkunle, A.T.J. and Ladejobi, T. A. 2006. Ethnobotanical and phytochemical studies on some species of Senna in Nigeria. African Journal of Biotechnology 5 (21):2020-2023.

Okwu, D.E. 2005. Phytochemicals, vitamins and mineral contents of two Nigerian medicinal plants. International Journal of Molecular Medicine and Advance Sciences 1(4): 375-381.

Okwu, D.E. and Okwu, M.E. 2004. Chemical composition of *Spondias mombin* Linn plant parts. Journal of Sustainable Agriculture and Environment 6(2): 140-147.

Omotayo, F.O. and Borokini, T.I. 2012. Comparative phytochemical and ethnomedicinal survey of selected medicinal plants in Nigeria. Scientific Research and Essays 7(9): 989-999.

Rabbani, G.H., Albert, M.J., Rahman, H. and Chowdhury, A. 1999. Short chain fatty acids inhibit fluid and electrolyte loss induced by cholera toxin proximal colon of rabbit *in vivo*. Digestive Disease Science 44: 1547-1553.

Saganuwan, A.S. and Gulumbe, M.L. 2006. Evaluation of *in vitro* antimicrobial activities and phytochemical constituents of Cassia occidentalis. Animal Research International 3: 566-569.

Scalbert, A. 1991. Antimicrobial properties of tannins. Phytochemistry 30: 3875-3883.

Shan, B., Cai, Y.Z., Brooks, J.D. and Corke, H. 2007. The *in vitro* antibacterial activity of dietary spices and medicinal herbs extracts. International Journal of Microbiology 117:112-119.

Shariff, Z.U. (2001). Modern herbal therapy for common ailments. Nature Pharmacy Series (Volume 1), Spectrum Books Limited, Ibadan, Nigeria in Association with Safari Books (Export) Limited, United Kingdom. pp. 9-84.

Sharma, N., Trikha, P., Athar, M. and Raisuddin, S. 2000. *In vitro* inhibition of carcinogen-induced mutagenicity by Cassia occidentalis and Emblica officinalis. Drug and Chemical Toxicology 23: 477-84.

Sivapriya, M., Dinesh, R., Ilarsha, R., Gowda, S.S.T. and Srinivas, L. 2011. Antibacterial activity of different extracts of Sundakai (Solanum torvum) fruit coat. International Journal of Biological Chemistry 5(1): 61-67.

Sofowora, A.E. 1993. Medicinal plants and traditional medicines in Africa. *2nd Edition*. Spectrum Books Limited, Ibadan, Nigeria. pp. 289.

Sundaram, M.M., Karthikeyan, K., Sudarsanam, D. and Brindha, P. 2010. Antimicrobial and anticancer studies on Euphorbia heterophylla. Journal of Pharmacy Research 3(9): 2332-2333.

Taylor, L.N.D. 2006. Database Entry for Ubos- Spondias mombin. pp. 1-5.

Tona, L., Cimanga, R.K., Mesia, K., Musuamba, C.T., De Bruyne, T., Apers, S., Hernans, N., Miert, S.V., Pieters, L., Totté, J. and Vlietinck, A.J. 2004. *In vitro* antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. Journal of Ethnopharmacology 93: 27-32.

Tona, L., Ngimbi, N.P., Tsakala, M., Mesia, K., Cimanga, K., Apers, S., De Bruyne, T., Pieters, L., Totté, J. and Vlietinck, A.J. 1999. Antimalarial activity of 20 crude extracts from nine african medicinal plants used in Kinshasa Congo. Journal of Ethnopharmacology 68: 193-203.

Torell, J., Cillard, J. and Cillard, P. 1986. Antioxidant activity of flavonoids and reactivity with peroxy radical. Biochemistry 25(2): 383-385.

Trease, G.E. and Evans, W.C. 1989. Pharmacognosy. Thirteenth Edition. Balliere Tindall, London. pp. 882.

Valarmathy, K., Gokulakrishnan, M., Kausar, M.S. and Paul, K. 2010. A study of antimicrobial activity of ethanolic extracts of various plant leaves against selected microbial species. International Journal of Pharmacological Sciences and Research 1(8): 293-295.

World Health Organization 2010. Cholera. Fact Sheet No. 107.

Wu, C., Chen, F., Wang, X., Kim, H.J., He, G.Q., Haley-Zitlin, V. and Huang, G. 2006. Antioxidant constituents in Feverfew (Tanacetum parthenium) extract and their chromatographic quantification. Food Chemistry 96: 220-227.

Yadav, J.P., Arya, V., Yadav, S., Panghal, M., Kumar, S. and Dhankhar, S. 2009. Cassia occidentalis: a review on its ethnobotany, phytochemical and pharmacological process. Fitoterapia 10: 1016.