International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 3 (2014) pp. 910-923**
http://www.ijcmas.com

Original Research Article

Preliminary studies on the antimicrobial effects and phytochemical studies of some Nigerian medicinal plants on some human pathogens

A.O.Daniels[1] and Malomo, Olu[2*]

[1]Department of Microbiology, University of Ado-Ekiti, P.M.B 5363, Ado Ekiti, Ekiti State. Nigeria.
[2]College of Food Sciences, Bells University of Technology,Ota, Nigeria
Corresponding author

A B S T R A C T

Five local medicinal plants used in folkloric treatment of skin infection were used against four bacteria and fungi that are not principal agents in skin diseases to test for their broad spectrum antimicrobial activities. These organisms are implicated in other diseases like wound infections, urinary tract infections, pulmonary infections, zygomycosis, mucomycosis, thyphoid, shigellosis, diarrhea, respiratory tract infections e.t.c. The plants include; Butyrosporum paradoxum Pseudocedrella kotschii, Cleorodendrum capitalum, Cassia occidentalis and Piliostigma reticulatum. Solvents such as ethanol, methanol, chloroform, ethyl acetate, N-hexane and sterile water were used to extract the active components of the plants. The ethanol extracts of B. paradoxum at concentrations of between 100mg/ml to 10mg/ml were active against salmonella typhorium and Klebsiella with zones of inhibition ranging from 24mm ±0.94 to 10mm ± 1.19. Ethanolic extract of Pseudocedralla kotschyii was active against Salmonella typhimorium, Klebsiella and pseudomonas aeruginosa with zones of inhibition ranging from 26mm ± 1.19 to 12mm ± 0.84. Ethanol extract of Cleorodendrum capitalum was active against Pseudomonas aeruginosa, Salmonella typhorium and shigella with zones of inhibition ranging from 22mm ± 0.89 to 12mm ± 0.94. The methanolic extract of this plant was also active against P. aeruginosa and S. typhorium with inhibition range of 22mm ± 1.19 and 12mm ± 0.89.Ethanolic extract of P reticulatum was also active against S. typhorium and Shigella with Inhibition zones of 18mm ± 1.12 and 14mm ± 0.89 at relatively high concentration of between 100mg/ml and 60mm/ml. The antifungal analyses also showed activity with B. paradoxum showing fungal inhibition with zones ranging from 20mm± 0.89 to 10mm± 1.19 against Sycephalastrum racemosum, Synnematum spp and Giberella saffulta. The ethanolic extract of P. kotchyii showed activity against S. racemoum and S. spp with inhibition zones of between 24mm± 0.89 to 12mm ± 0.84. Ethanol extract of C. occidentalis was active against all fungal strains including Cumminghamella elegans with zones ranging from 22mm ± 1.19 to 14mm± 0.89. The methanol extract of C. capitalum was active against C. elegans with zones ranging from 20mm± 0.89 to 12mm ±1.12 while the ethanolic extract was active against G. saffulta with zones ranging from 18mm ±1.19 to 12mm. ± 0.84. They ethanolic extract of P. reticulatum showed static activity against S. racemosum but cidal activity against C. elegans with zones of inhibition raging from 20mm ± 0.84 to 14mm± 0.89.Phytochemical screening showed the presence of saponin, tannin, alkaloid, cardiac glycoside, phlombatannin, flavonoids e.t.c. Negative and positive control using standard antibiotics showed most of the test organisms are resistant to the antibiotics. Pseudomonas aeruginosa was sensitive to Nitrofnrantoin and gentamycin. S. racemosum was sensitive to ciprofloxacin, pefloxacin and ofloxacin G. saffulta was sensitive to ofloxacin.

K e y w o r d s

Inhibition range; skin infections; antimicrobial activities; phytochemical screening, herbal medicines

Introduction

Water is not a commercial product but, supported by modern medicine and scientific research. (Ferris, 2013). In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the

emergence of new bacterial strains that are multi-resistant (WHO, 2001, Aibinu et al., 2003; Aibinu *et al.*, 2004). The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality (Williams, 2000). Therefore, there is a need to look for substances from other sources with proven antimicrobial activity. Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs (Pretorius *et al.,* 2003, Moreillion et al., 2005).

Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life-style related disorders (Agyare *et al.*, 2009). The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs into the African continent. Herbal medicine has been widely used and formed an integral part of primary health care in China, Ethiopia, Argentina and Papau New Guinea (Akinyemi *et al.*, 2005).

Herbal remedies have a therapeutic effect and are acceptable interventions for diseases and symptoms. Interestingly, demand for medicinal plants is progressively rising in industrialized nations as it is in developing countries (Abere *et al.*, 2010). The World Health Organization (WHO) estimated that about 80% of the developing world's population meets their primary health care needs through traditional medicine (Abere *et al.*, 2010; Calixto, 2000; Green, 2000; Jadeja *et al.*, 2011). About 25% of drugs prescribed and dispensed in the United States contain at least one active component derived from plant matter.

While some are synthesized to mimic a natural plant compound, others are made from plant extracts. It has been estimated that one out of every three people in the United States had tried at least one form of alternative medicine (Eisenberg *et al.*, 1993).

Within the last few decades, many plants have been screened for their biological and pharmacological properties by researchers. These steps are continually being taken to study the intrinsic worth of traditional medicine in the light of modern science with the scrutiny aimed at adopting effectively beneficial medical practice and discouraging harmful ones (Abere *et al.*, 2010). Note worthily, components of herbs serve as starting materials for a number of old and new pharmaceutical products. About 25% of modern pharmaceutical drugs have botanical origins.

Butyrospermum paradoxum is a small deciduous tree with a corky bark, oblong leaves and ellipsoidal oily seed. It belongs to the family sapotacea, Genus Botyrospermum. The seed is used traditionally as cooking fat, illuminant, medicinal ointment, hairdressing and for soap. All parts of the tree are used in medicine, for skin diseases including leprosy, dermatitis and eczema, to protect from sunburn, as the oil can counter harmful ultra-violet rays from the sun, and also soothe sunburn. It is also used for wounds and to massage into stiff joints, as well as to treat sore and injuries of animals. The roots are used for cleaning the teeth and oral health (Ogunwande et al., 2001).

C occidentalis is a small tree that grow 5 – 8cm of South America, including the Amazon. It is in the some genus as Senna and sometimes called coffee senna." Seeds pods long are sometimes roasted and made a coffee-like beverage. The cassia genus comprises some species of trees, shrubs, vines and herbs with numerous species growing in the South American rainforest, and tropics. Many species have been medicinally, and these tropical plants have a history in natural medicine as purgative and laxatives . The main plant chemicals in C. occidentalis include: achrosine, emodin, anthraquinones, anthrones, apigenin, sitosterols, tannins and xanthones.

Traditionally, its roots, leaves, flowers and seeds are used as laxative and purgative (Todd, 1967). It is a vermifuge, anticonvulsant and used against chicken pox (Mann, 2003). Other uses include febrifuge, extrusion of guinea worm (Iwu, 1993) and black quarter (Ndi et al., 2000). Previous studies have shown that its leaves exhibited in-vitro antibacterial, antimalarial and antihepatotoxic properties (Gasquet, 1993; Percez, 1994; Saraf, 1994). Seeds are brewed into a coffee like beverage for asthma and the flower infusion is used for bronchitis in the Peruvian Amazon (Akinloye et al., 2003).

Cleorodendrum capitalum

Clerodendron capitatum (Willd) (family: verbenaceae) is locally named as Gung and used traditionally to treat erectile dysfunction(Abdelwahab et al., 2011). It is also used in the folkloric treatment of type II diabetes (anon, 2009),

Piliostigma reticulatum **(**DL.) Hochst. (common name; Yoruba: 'abafin', Hausa: 'kalgo', Igbo: okpo atu') belongs to the family Leguminosae - Caesalpiniaceae and is found in the savannah region of Nigeria. It is a tree, occurring up to 30ft in height with an evergreen, dense spreading crown (Keay, 1989).

It is used traditionally in the treatment of diarrhea. Tea from the leaves to treat colds, bark is astringent and used against diarrhoea and dysentery ; leaves and bark have haemostatic and antiseptic properties, cures also ulcers, boils, wounds and syphilitic cancer. Other medical uses are against coughs, bronchitis, malaria, hepato-biliary ailments, hydropsy, sterility, rachitis and kwashiorkor.

Pseudocedrella kotchyii Harms is a single specie genus belonging to the family Meliaceae widely distributed in sub Sahara zone of Central Africa. The roots and leaves are used medicinally in Nigeria for rheumatim and other diseases. The bitter bark is used in Congo as infusion for gastrointestinal, febrile and rheumatic condition (Haruna et al., 2008).A decoction of the root-bark and leaves is used as sitz bath for pile.

Materials and Methods

Plant sampling and preparation

Plants extracts were prepared by suspending 40gm of pulverized sample in 100mls of ethanol, ethyl acetate and distilled water. The solutions were allowed to sand for 120hr (5days) and then filtered using Whatman No 1 filter paper. The filtrates were allowed to evaporate to dryness and then reconstituted using DMSO.

The extracts were diluted serially to give stock percentage of 100mg/ml, 90mg/ml, 80mg/ml, down to 25mg/ml. These serial

dilutions were used for the agar well antimicrobial test**.**

Microorganisms

The microorganisms used in this study were collected from the microbiology department of Obafemi Awolowo University, Ile - Ife and biochemical tests were carried out authenticate the identification of the microorganisms. These organisms include; *Pseudomonas aeruginosa, Klebsiella aerogenes, Shigella and Salmonella typhorium. Fungi include; Cunminghamella elegans, Syncephalastrum racemosum, Synnematium spp, Gibellula saffulta***.**

Antimicrobial test

Broth cultures of bacteria were prepared by suspending a loopful of bacterial culture from slants into nutrient broth and incubated for four hours to obtain a culture compared to Macfarland's constant of $0.5x10^8$. The 4hrs broth cultures were used to seed agar plates, prepared by pouring about 20mls of molten nutrient agar into sterile petridishes by pour plate method. The plates were allowed to set and the agar well diffusion method was used to carry out the antimicrobial test.

Positive and negative control

Standard antibiotics were tested against test organisms to detect the susceptibility or resistance of microorganisms to the antibiotic disc to justify the need for new antimicrobial. Commercial antibiotic discs were used in this work and the agar disc diffusion method of Kirby- Bauer (1966) was employed. Standard antibiotic discs used include; Amoxylin, Ofloxacin, Streptomycin, Chloramphenicol, Ceftriazone, Gentamycin, Pefloxacin, Cotrimoxazole, Ciprofloxacin and Erythromycin as the negative control and Augmentin, Ceftriazone, Nitrofurantoin, Gentamycin, Cotrimoxazole, Amoxylin, Ciprofloxacin, Tetracyline, Pefloxacin Were used for the negative control.

Biochemical test

Biochemical tests were carried out to confirm the identification and the physiological properties of the test organisms. Biochemical tests carried out include; Catalase, Motility, Gram staining, Indole test Methyl red, test, Vogeus preskaur Citrate test, Triple sugar iron test, Urease test, Oxidase, Carbohydrate Fermentation test (S/G/L)**.**

Phytochemical screening

Qualitative Analysis of Phytochemicals

Chemical tests performed in the screening and identification of phytochemical constituents in the tested medicinal plants were carried out in extracts as well as powder specimens using the standard procedures as described by Sofowara (1993); Trease and Evans (1989); Harborne (1998).

Preparation of reagents

Mayer's reagent: 0.355 g of mercuric chloride was dissolved in 60 ml of distilled water. 5.0g of potassium iodide was dissolved in 20 ml of distilled water. Both solutions were mixed and volume was raised to 100 ml with distilled water.

Dragendorff's reagent: Solution A: 1.7 g of basic bismuth nitrate and 20 g of tartaric acid were dissolved in 80 ml of distilled water. Solution B: 16 g of potassium iodide was dissolved in 40 ml

of distilled water. Both solutions (A and B) were mixed in1:1 ratio.

Test for alkaloids

About 0.5 to 0.6 g of the methanolic plant extract was mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate were treated separately with both reagents (Mayer's and Dragendorff's), after which it was observed whether the alkaloids were present or absent in the turbidity and/or precipitate formation.

Test for steroids

About 0.5 g of the methanolic extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids.

Test for terpenoids

An aliquot 0.5 ml of methanolic extract was mixed with 2 ml of $CHCl_3$ in a test tube. 3 ml of concentrated H2SO4 was carefully added to the mixture to form a layer. An interface with a reddish brown coloration was formed in the presence of terpenoids, as positive result.

Test for flavonoids

To the substance in alcohol, a few magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for five minutes. Red coloration identifies the presence of flavonoids (Shinado's test).

Test for tannins

The 0.5 g of powdered sample of each medicinal plant leaves was boiled in 20 ml of water in a test tube and then filtered. The filtration method used here was the normal method, which includes a flask and filter paper. The 0.1% FeCl3 was added to the filtered samples and observed for brownish green or a blue black coloration, which showed the presence of tannins.

Quantitative analysis

Extraction and Estimation of Phenolics (Price et al., 1980)

25µl of acetone and 60µl of ferric ammonium sulphate were added to plant extract and kept at room temperature for 20 minutes. To this, 60µl of potassium ferricyanide was added and the absorbance was measured at 720 nm after 20 minutes by incubation at room temperature. Quercetin was used as a standard and the total phenolics were expressed as µg/gm of extract.

Extraction of Flavonoids (Harborne, 1975)

Flavonoids are generally present as their glycosides. They are hydrolyzed and free flavonoids were assayed. The extract (1ml) was mixed with 1ml of 2% methanolic $AlCl_3$ and the absorbance was measured at 430 nm. Quercetin dissolved in methanol in the range of 100-500µg was used as standard

Results and Discussion

The biochemical test carried out aided in the confirmation of the test organisms. The result of the biochemical test is recorded in table 1.

The result of the antimicrobial test using the plant extracts is shown in table 2. The ethanolic extracts of the plants showed

more activity as compared with other solvent extracts. However, methanol extract, chloroform extract and ethyl acetate extracts of the plants were active against some bacteria and fungi. *B. paradoxum, C. occidentalis* and *P. reticulatum* were not active against *P. aeruginosa* (Table 2). Only the ethanol extracts of *B.paradoxum* and *P .kotchyii* were active against *Klebsiella* (Table 2). Antifungal effect of plants was not appreciable as very few of the plants showed activities (Table 3). Ethanol extract of *P. reticulatum* was showed cidal activity against *S. racemosum* (Table 3).

Positive and negative control carried out using standard antibiotic discs showed high resistance of microorganisms to the antibiotics used. Only *Pseudomonas aeruginosa* was sensitive to nitrofurantoin and gentamycin.(Table 7and 8). However, fungi showed higher level of activities against the antibiotics. S. racemosum was sensitive to ofloxacin, ciprofloxacin and pefloxacin while *G. saffulta* was sensitive to ofloxacin (Table 9 and 10).

Several medicinal plants are used traditionally for the treatment of several microbial infections. There is a call by the WHO on developing countries to improve health delivery by the primary health care approach; meanwhile, the use of herbal medication is recognized as an integral part of the primary health care system.

An important aspect of evaluating herbal medications should be a screening for possible broad spectrum ability of the plants.

The antimicrobial screening of the plants showed considerable activities. This could be attributed to the presence of phytochemicals such as saponins, flavonoid, tannins, streroid, glycosides, anthroquinones and alakaloids.(Tables 4,5 and 6).

The ethanol extract of *Butyrospermum paradoxum* showed antibacterial activities against Salmonella typhi and Klebsiella pneumonia. The activity against Klebsiella showed an inhibition zone of 24mm at 100mg/ml conc of extract. This shows a high antimicrobial property against the organism. Although *B. paradoxum* is used primarily for the treatment of skin conditions like leprosy and dermatitis, it's activity against Salmonella (16mm – 10mm) and *Klebsiella* (224mm -14mm) which are implicated in typhoid fever, food poisoning and bacteria cystitis (UTI) respectively shows the broad spectrum activity of the plant.

The antifungal test showed *Syncephalastrum racemosum* (20mm - 14mm), *Synnematium* spp (24mm -16mm) and Gibellula sulfatta (18mm -10mm) to be sensitive to B paradoxum with the corresponding zones of inhibition. S. racemosum is implicated in nail diseases (Pavlovic and Bulaji,2006) and intra abdominal zygomycosis (Schlebush and David, 2005).

Ethanol extract of Pseudocedrella kotschyii was active against S. racemosum and Gibellua saffulta with zones of inhibition range of 22mm to 12mm. The antibacterial test also revealed the ethanol extract of the plant to be active against Pseudomonas aeruginosa, Salmonella typhi and klebsiella which is an intestinal organism with zones of inhibition ranging from 26mm to 12mm. This plant is used in the traditional treatment of rheumatism and pile. With the result recorded in tables

Table.1 Biochemical and physiological test.

S/N	Tests	*Salmonella*	*Shigella*	*Klebsiella*	*Pseudomonas*
1.	Catalase	+	-	+	+
2.	Motility	+	-	-	-/rod
3.	Gram staining	-	-	+	_
4.	Indiole test	-		-	-
5.	Methyl red test	+	-	-	-
6.	Vogeus preskaur Citrate test	-	-	-	+
7.	Triple sugar iron test	Alk/acid butt/H_2S /no gas	Alk red, acidic butt, no gas, no H_2S	Acid	Acid
8.	Urease test	-	-	-	+
9	Oxidase	-	-	-	+
10	Carbohydrate Fermentation test				
	Sucrose	no acid	-	-	-ve
	Glucose	acid and gas	-	+, acid,	-ve
	Lactose	-ve, no acid	-ve	Acid and gas	-ve

two and three, the broad spectrum activites of the plant against both bacteria and fungi has been established. The Ethanol and methanol extracts of Cassia occidentalis were also active against Salmonella while the water extract was active against *Shigella*. Antifungal test showed all the fungal strains to be sensitive to the plant at high concentration. This plants is used as laxative and purgative. And anticonvulsant and used against chicken pox. This plant also exhibits broad spectrum activities.

Piliostigma reticulatum , a plant noted for its medicinal treatment of diarrheoa, dysentery , ulcer, wound and syphilitic ulcer showed activity against some of the test organisms with the ethanol extract inhibiting *Salmonella*, *Shigella* and *Klebsiella* with zones of inhibition running from 18mm -14mm, methanol extract showing same zone of inhibition the ethyl acetate extract showing the highest zone of inhibition from 30mm -14mm.but the plant did not inhibit *Pseudomonas aeruginosa*. This negates the claim that the plant is used in the treatment of wound since *P. aeruginosa* is an commonly isolated from wound .However the plant showed light antifungal activity (Table 3). This can further be investigated for its use as an anifungal.

Cleorodendron capitalum is use traditionally to treat diabetes II (Anon, 2003) and erectile disfunction (Abdulwahab et al, 2011) has antimicrobial activities with the ethanol extract inhibiting *P. aeruginosa*, *S. typhi*, and *Shigella* with zones of inhibition of 26mm -12mm, and the methanol extract showing inhibition against P. aeruginosa and S. typhii with zones ranging from 22mm-12mm. It showed antifungal effect on two fungi (Table 3) also confirming the broad spectrum activity of the plant.

Table.2 Antibacterial screening of plant extracts

Extracts	*Pseudomonas*					*Salmonella*					*Shigella*					*Klebsiella*				
	100	60	40	20	10	100	60	40	20	10	100	60	40	20	10	100	60	40	20	10
B. paradoxum in Ethanol	-	-	-	-	-	16	14	12	10	-	-	-	-	-	-	24	22	20	16	14.
B. paradoxum in methanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B.paradoxum in water	-	-	-	-	-	16	14	12	10	-	-	-	-	-	-	-	-	-	-	-
B. paradoxum in E.A.	-	-	-	-	-	--	-	-	-	-	-	-	-	-	-	-	-	-	-	=
P. kotchyii in ethanol	18	16	14	12	-	26	24	20	16	14	-	-	-	-	-	20	18	16	14	12
P. kotchyii in methanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--	-	-	-	-	-
P. kotchyii in water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. kotchyii in E.A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. occidentalis in ethanol	-	-	-	-	-	18	16	14	12	-	-	-	-	-	-	-	-	-	-	-
C. occidentalis in methanol	-	-	-	-	-	22	18	16	14	12	-	-	-	-	-	-	--	-	-	-
C. occidentalis in water	-	-	-	-	-	-	--	-	-	-	18	16	12	10	-	-	-	-	-	-
C . occindentalis in E.A.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. capitalum in ethanol	18	16	14	12	-	22	20	16	12	-	26	24	22	20	16	-	-	-	-	-
C.capitalum in methanol	22	20	16	14	-	20	16	12	-	-	-	-	-	-	-	-	-	-	-	-
C. capitalum in water	-	-	-	-	-	-	-=	-	-	-	-	-	-	-	-	-	-	-	-	-
C. capitalum in E.A																-	-	-	-	-
P. reticulatum in ethanol	-	-	-	-	-	18	16	-	-	-	14	-	-	-	-	-	-	-	-	-
P. reticulatum in methanol	-	-	-	-	-	18	16	14	-	-	-	-	-	-	-	-	-	-	-	-
P. reticulatum in water.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P.reticulatim in E.A.	-	-	-	-	-	30	26	24	20	18	26	24	22	20	18	16	14	-	-	-

Table.3 Antifungal effects of plant extracts

Plants	**2fsub1**					**2c/c10sub2**					**D10sub 2**					**EY/C3**				
	100	60	40	20	10	100	60	40	20	10	100	60	40	20	10	100	60	40	20	10
B. paradoxum in Ethanol	20	18	16	14	-	24	22	20	18	16	18	16	14	12	10	-	-	-	-	-
B. paradoxum in methanol	-	-	-	-	-	-	-	-	-	-	-	-	-	--	-	-	-	-	-	-
-B.paradoxum in water						--	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B. paradoxum in E.A.						-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. kotchyii in ethanol	22	20	16	12	-	-	-	-	-	-	18	16	14	12	-	-	-	-	-	-
P. kotchyii in methanol					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. kotchyii in water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--	-	-	-	-
P. kotchyii in E.A	-	-	-	--	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. occidentalis in ethanol	24	22	18	--	-	22	20	16	12	10	20	18	16	14	12	20	18	16	-	-
C. occidentalis in methanol						-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. occidentalis in water	--	-	-	-	-	-	-	-	-	-	-	-	-	-	--	-	-	-	-	-
C . occindentalis in E.A.	-	-	-	--	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. capitalum in ethanol	-	-	-	-	-	-	-	-	-	-	18	16	14	12	-	-	-	-	-	-
C.capitalum in methanoL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	18	16	14	12	
C. capitalum in water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--	-	-	-
C. capitalum in E.A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. reticulatum in ethanol	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. reticulatum in methanol###	20	18	16	14	-	-	-	-	-	-	-	-	-	-	-	20	18	16	14	-
P. reticulatum in water.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P.reticulatim in E.A.	20	-	-	-	-	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table.4 Phytochemical screening of plants (Ethanol extracts)

Plants	Saponin	flavonoid	Tannin	Glycosides	Phenols	Steroids	Phlombantanins	Anthroquinone	Alkaloids
C.occidentalis	+	-	+	+	+			+	-
B. paradoxum	+	-	-	+				+	
C.capitalum	+	+	+	+	-	+	+	+	+
P.reticulatum	+	+	+	+	+			+	-
P.kotchyii	+	+	+	+	-	-	-		

Table.5 Phtyochemical screening of plants (Methanol extracts)

Plants	Saponin	flavonoid	Tannin	Glycosides	Phenols	Steroids	Phlombantanins	Anthroquinone	Alkaloids
C.occidentalis	+	-	+	+				+	-
B. paradoxum	+	-	-	+					
C.capitalum									
P.reticulatum	+	+	+	+	+	-	-	-	-
P.kotchyii	+	+	+	+	-	-	-	-	-

Table.6 Screening of plants (Water extacts)

Plants	Saponin	flavonoid	Tannin	Glycosides	Phenols	Steroids	Phlombantanins	Anthroquinone	Alkaloids
C.occidentalis	+	-	+	+				+	-
B. paradoxum									
C.capitalum	+	+	+	+	-	+	+	*	+
P.reticulatum									
P.kotchyii									

Table.7 Bacterial - positive control

CODE	***AB***	***Salmonella***	***Pseudomonas***	***Shigella***	***K.aerogenes***
AMX	Amoxylin	-	-	-	-
OFL	Ofloxacin	-	-	-	-
STR	Streptomycin	-	-	-	-
CHL	Chloramphenicol	-	-	-	-
CFF	Ceftriazone	-	-	-	-
GEN	Gentamycin	-	-	-	-
PFL	Pefloxacin	-	-	-	-
COT	Cotrimoxazole	-	-	-	-
CPX	Ciprofloxacin	-	-	-	-
ERY	Erythromycin	-	-	-	-

Table.8 Bacteria - negative control

CODE	**AB**	***Salmonella***	***Pseudomonas***	***Shigella***	***K.aerogenes***
AUG	Augmentin	-	-	-	-
CRO	Ceftriazone.	-	-	-	-
NIT	Nitrofurantoin	-	14mm	-	-
GEN	Gentamycin	-	18mm	-	-
COT	Cotrimoxazole	-	-	-	-
OFL	Ofloxacin	-	-	-	-
AMX	Amoxycillin	-	-	-	-
CPX	Ciprofloxacin	-	-	-	-
TET	Tetracycline	-	-	-	-
PFX	Pefloxacin	-	-	-	-

Table.9 Positive control - Fungi

CODE	AB	2fsub 1	Ey/c 3	D10 SUB 2	2 C/C10SUB 1
AMX	Amoxylin	-	-	-	-
OFL	Ofloxacin	-	-	-	-
STR	Streptomycin	-	-	-	-
CHL	Chloramphenicol	-	-	-	-
CFF	Ceftriazone	-	-	-	-
GEN	Gentamycin	-	-	-	-
PFL	Pefloxacin	-	-	-	-
COT	Cotrimoxazole	-	-	-	-
CPX	Ciprofloxacin	-	-	-	-
ERY	Erythromycin	-	-	-	-

Key; 1. 2fsub1- Syncephalastrum racemosum; 2.EY/C-.Cunninghamella elegans
3. 2c/c10 sub2-Synnematium spp ; 4. D10sub2-Gibellula salffata

Table.10 Fungi - negative control

CODE	AB	2FSUB1	EY/C3	D10SUB2	2 C/C10SUB 1
AUG	Augmentin	-	-	-	-
CRO	Ceftriazone.	-	-	-	-
NIT	Nitrofurantoin	-	-	-	-
CGEN	Gentamycin	-	-	-	-
COT	Cotrimoxazole	-	-	-	-
OFL	Ofloxacin	22mm	-	16mm	-
AMX	Amoxycillin	-	-	-	-
CPX	Ciprofloxacin	20mm	-	-	-
TET	Tetracycline	-	-	-	-
PFX	Pefloxacin	12mm	-	-	-

Key; 1. 2fsub1- Syncephalastrum racemosum; 2.EY/C-.Cunninghamella elegans
3. 2c/c10 sub2-Synnematium spp ; 4. D10sub2-Gibellula salffata

The comparative antibiotic screening using conventional antibiotics revealed most of the bacteria strains to be resistant to the antibiotics except Pseudomonas aeruginosa that was sensitive to gentamycin and nitrofurantoin. This result further confirms the emergence of resistance of microorganisms to current antimicrobials and also support the need for a vigorous search for substitute antimicrobial of which such plants as used in this work serve as a qualified candidate plants in drug production. However, more fungal strains show more level of sensitivity to the antibiotic used, *Syncephalostrum racemosum* was sensitive to ofloxacin, ciprofloxacin and pefloxacin . This could be as a of the fact these organisms are not common clinical organisms and might not have been previously exposed to the antibiotics and might not have experienced genetic change.

Phytochemical screening showed the plants to contain active phytocompounds like alkaloid, tannin, saponin, flavonoids, glycosides, phlombatanins and anthroquinones.

Herbal drugs contain unique constituents which differs from one herb to another, hence the type and extent of their medicinal property also differs. This explains the different antimicrobial level and strength of each plant. Phytochemicals are generally known to be part of bioactive components of plants have shown to possess medicinal and antimicrobial values as well as exhibiting physiological properties (Di Vincenzo, 2005). Flavonoid is used in medicine as antimicrobial, anti inflammatory and anti oxidant (Evans, 1996).

This work has shown that the plants used in this study can further be subjected to characterization and toxicity test so as to be included in the data base as a candidate for drug production.

References

Aibinu I E, Ohaegbulam V C, Adenipekun E A, Ogunsola F T, Odugbemi T O, Mee B J. Extended-Spectrum Beta-Lactamase Enzymes in Clinical Isolates of *Enterobacter* species from Lagos, Nigeria. Journal of Clinical Microbiology. 2003;415:2197–2200.

Aibinu I, Adenipekun E, Odugbemi T. Emergence of Quinolone Resistance amongst *Escherichia coli* strains isolated from clinical infections in some Lagos State Hospitals in Nigeria. Nigerian Journal of Health and Biomedical Science. 2004;32:73–78.

Abdulwahab S.I, Mohammed, A.H., Mohammed O.Y., Oall, M., Taha,M.M, Mohan, S. Noodin, M.I, Mustapha, M.R, Alkharfy, K.M., 2011.Erectogenic effects of C. capitalum. Evidence based complimentary and Alternative medicine vol, 2012, ID 137386,PG 123-129.

Abere, T. A., Okoto, P. E., Agoreyo, F. O. 2010. Antidiarrhoea and toxicological evaluation of the leaf extract of Dissotis rotundifolia Triana Melastomataceae. BMC Complement Altern Med 10: 71.

Akinyemi, K. O., Oladapo, O., Okwara, C. E., Ibe, C. C., Fasure, K. A. 2005. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant Staphylococcus aureus activity. BMC Complement Altern Med 5: 6.

Akinloye, O. A., Ahmed, A. S., Ajagbona, O. P. and Olorede, B. R. 2003. Some biochemical effects of various doses of aqueous seed extracts of Cassia occidentalis in rabbits. Bioscience Research Communication, 151: 85 – 90.

Anonymous, 2009. www.research. net/publications, 157.

Agyare, C., Asase, A., Lechtenberg, M., Niehues, M., Deters, A., Hensel, A. 2009. An ethnopharmacological survey and in vitro confirmation of ethnopharmacological use of medicinal plants used for wound healing in Bosomtwi-Atwima-Kwanwoma area, Ghana. J Ethno, pharmacol 1253: 393-403.

Buer ,A.W. Kirby, M.M., Sherris, J.C and Turk, M. 1966. Antibiotic susceptibility testing by standardization single dose method. American Journal of Clinical pathology 36;493-496.

Calixto, J. B. 2000. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines phytotherapeutic agents. Braz J Med Biol Res 33: 179-189.

Eisenberg, D. M., Kessler, R. C., Foster, C., Norlock, F. E., Calkins, D. R., Delbanco, T. L. 1993. Unconventional medicine in the United States. Prevalence, costs, and patterns of use. N Engl J Med 3284: 246-252.

GASQUET, M. 1993. Evaluation in-vitro and in-vivo of traditional antimalarial plants. Fitaterapia645: 423 – 424.

Green, E. C. 2000. The WHO forum on traditional medicine in health systems, Harare, Zimbabwe, February 14-18, 2000. *J Altern Complement Med* **6**5**:** 379-382.

IWU, M. M. 1993. Catalogue of major medicinal plants. Handbook of African Medicinal plants CRC Press, London.

Jadeja, R. N., Thounaojam, M. C., Ansarullah, Jadav, S. V., Patel, M. D., Patel, D. K., Salunke, S. P., Padate, G. S., Devkar, R. V., Ramachandran, A. V. 2011. Toxicological evaluation and hepatoprotective potential of Clerodendron glandulosum.Coleb leaf

extract. *Hum Exp Toxicol* 301**:** 63-70.

Muhammadu H.J 1990. African Traditional Medicine; A case study of Hausa Medicinal plants and therapy. 1st edition Gaskiya Corporation Ltd. Zaria pp. 20-21.

Moreillion P, Que YA, Glauser MP. *Staphylococcus aureus* Including Staphyloccal Toxic shock In: Mandell GL, Bennett JE, Dolin R, editors. 'Principles and Practice of Infectious diseases'. 6th ed. Vol. 2. Pennyslyvania: Published by Churchill livingstone; 2005. pp. 2333–2339

National Research Council NRC 2008. Lost crops of Africa. Volume III: Fruits. National Academic press pp. 1-5.

Ndi, C., Mumah, E. T., Ndokwo, K. J., MFI, A. N. Nbenkum, T., Asah, H., Ekue, F. N. and Toyong, J. N. 2000. Ethno veterinary plants used in the North West province of Cameroon. Pages 154 – 161. In: Ethnoveterinary practices research and development. Proceeding of an International Workshop on Ehnoveterinary Practices, held 14 – 18 August 2000, Kaduna Nigeria.

Ogunwande A.I., Bello, .M.O. Olawore, N.O 2001. Phytochemical and antimicrobial studies of B. paradoxum. Fitoterapia,72,1 54-56.

Pavlovic M.D., Bulajic, N, 2006. Great toeanail onychomycosis caused by S. racemosum. Dermatology on line J.12 1;7.

Perzec, C. 1994. In-vitro anti-bacterial activity of argentine folk medicinal plants against salla typhi. Journal o Ethnopharmacology, 441: 41 – 46.

Pretorius JC, Magama S, Zietsman PC. Growth inhibition of plant pathogenic bacteria and fungi by extracts from selected South African plant species. South African Journal of Botany. 2003;20:188–192.

Saraf l Mone, S. 1994. Anti-hepatotoxic activity of cassia occidentalis. International Journal of Pharmacology, 322: 178 – 183.

Schclubush, S. and David,F. M, 2005. Intraabominal zycomycosis. Journal of Clinical Microbiology, 43 11. 582-5827.

Todd, R. G. 1967. Senna and other purgatives. Pages 1266 – 1267. In: Extra pharmacopoeia Martindale, 20th Edition, W. B. Saunders, London.

Todd, R. G. 1967. Senna and other purgatives. Pages 1266 – 1267. In: Extra pharmacopoeia Martindale, 20th Edition, W. B. Saunders, London.

Williams R. Antimicrobial resistance a global threat. Essential Drug Monitor. 2000;28–29:1.