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Archives of Applied Science Research, 2012, 4 (5):1974-1990
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ISSN 0975-508X
CODEN (USA) AASRC9

Phytochemical and Antibacterial Evaluation of Selected Locally Produced Herbal Medicines Sold in Calabar, Nigeria

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ABSTRACT

Ten common herbal medicines sold in Calabar Metropolis were evaluated for their phytochemical constituents, possible contaminants and antibacterial properties using standard microbiological methods. A total of twelve (12) different bacteria and five (5) fungi were isolated from the selected herbal medicines with Penicillium spp. and Candida spp. having the highest frequency of occurrence 33.33% (7/21), followed by Bacillus spp. 23.81% (5/21). Aspergillus spp., Mucor spp. and Proteus spp., all had a frequency of occurrence of 14.28% (3/21). Flavobacterium spp., Klebsiella spp. and Staphylococcus aureus had a frequency of occurrence of 9.52% (2/21). Escherichia coli, Enterobacter spp., Fusarium spp., Lactobacillus spp., Micrococcus spp., Pseudomonas spp., Serratia spp. and Staphylococcus epidermidis all had a frequency of occurrence of 4.76% (1/21). The mean total viable bacteria count for UMU was 27.7 $\pm$ 3.39 x 10^9 $cfuml^{-1}$, UAE 24.5 $\pm$ 4.98 x 10^8 $cfuml^{-1}$. The count for others was within the acceptable limits. The phytochemical screening of the herbal medicines showed reducing compounds and polyphenol to be present in much excess amount (+++) in 50% and 30% of the sampled herbal medicines respectively. Alkaloids were present in excess amount (++) in 70% of the herbal medicines. Cardiac glycosides were present (+) in 90% of the herbal medicines sampled, while saponin was absent (-) in 80% of the herbal medicines sampled. Tanin was present in 60% of the selected herbal medicine. Phlobatanins, triperpenes, anthraquinones and hydroxylmethyl anthraquinones were absent (-) in 80%, 70%, 90% and 80% respectively of the herbal medicines sampled. The frequency of susceptibility of the test bacteria to herbal medicines was: Klebsiella pneumonia, Pseudomonas aeruginosa and Staphylococcus aureus 70%, Salmonella typhii and E. coli 60%, Proteus vulgaris 40%, Streptococcus faecalis 20% and Micrococcus spp. 10%. AAP could not inhibit any of the test bacteria while DAH herbal remedy was effective against all the test bacteria with zones of inhibition ranging from 9.50 to 20.88 mm. The MIC of the various herbal medicines was: CNB, EPL and UMU $\geq$1000$mgml^{-1}$, GBM and AAP 625$mgml^{-1}$, OCB and UAE 565$mgml^{-1}$, DAH herbal remedy and AMB 95$mgml^{-1}$ and NH2 herbal remedy 535$mgml^{-1}$. The MBC for the herbal medicines was 1125$mgml^{-1}$ for NH2 herbal remedy and GBM; 1250$mgml^{-1}$ for OCB, UAE and AAP. That of DAH herbal remedy and AMB was 200$mgml^{-1}$ and 300$mgml^{-1}$ respectively while that of CNB, EPL and UMU was $\geq$2000$mgml^{-1}$. This result reveals a high level of bacteria and fungi contaminants in herbal medicines sold in Calabar and makes need for intervention.

Key words: Phytochemical, herbal medicine, Calabar metropolis

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INTRODUCTION

Phytochemical comes from the Greek word "phyto" for plant. It refers to every naturally occurring chemical present in plants. Plants are also the source of many modern pharmaceuticals (drugs). It is estimated that approximately one quarter drug contain plant extract or active ingredients obtained from plant substances [17].

Plants and herbs have been used by man to cure diseases and heal injuries since time immemorial. In recent years, renewed interest has been shown in the use of medicinal plants and scientific studies are been designed to explain

some of the curative phenomena associated with traditional herbal remedies. Most drugs utilized by people all over the world are of plant origin [19] [12].

Herbal therapy is widely accepted and used as an alternative in the prevention and treatment of physical and mental disorders as well as infectious diseases and antisocial behaviours attributable to spiritual causes. Due to its intrinsic qualities, unique and holistic approaches as well as its accessibility and affordability, it continues to be the best alternative health care available for the majority of the global population, particularly for those in the rural areas of developing countries [15].

Among the successes achieved by herbal medicine is that many drugs in clinical use today were discovered from the way plants were used in traditional communities. Examples include quinine which was discovered from the way traditional communities in South America especially Peru, Columbia and Bolivia, used plant species of the genus *Cinchona* in managing fevers. Similarly, taxol is a modern day therapy for ovarian cancer obtained from *Taxus brevifolia* which was a traditional medicinal plant in British Columbia [10] [18].

In addition, herbal therapy is also seen as a panacea to the problems associated with drug resistance among infective agents. Mboya (2003) asserted that with increasing resistance of microorganisms associated with infectious diseases, and increasing environmental pollution which constitutes selective pressure on microorganisms, herbal medicine provides alternative sources for new drugs [13]. Thus emphasis is now being laid on traditional medicine as an alternative to orthodox medicine more than ever before. This is especially true in developing countries like Nigeria.

In Nigeria, the use of herbal medicine dates back to the earliest history of mankind, as in other cultures worldwide. Prior to the coming of orthodox medicine, most people relied totally on the use of traditional means for all their healthcare needs, these include the use of herbal, animal and mineral based herbal medicines often laced with spiritual ingredients such as incantations [16][12].

Presently, much research is been carried out by pharmaceutical industries in developed countries, thus if this kind of work is not done in Nigeria, it means that huge sums of money will be spent by our government in future to re-import our own plant-derived drugs and plant extracts.

MATERIALS AND METHODS

Study Site and Sample Collection

This study was carried out in Calabar, Cross River State, Nigeria. Cross River State is a coastal state in south-south region of Nigeria. It shares boundaries with Benue State to the North, Enugu and Abia State to the West, to the East by Cameroun and to the South by Akwa- Ibom State and the Atlantic Ocean. Calabar is located approximately between longitude 80 19^{l}E and 80 21^{l}E and latitude 40 55^{l}N and 40 58^{l}N. A total of ten (10) herbal medicines were randomly selected and purchased for the research from different locations in Calabar and codes such as DAH, CNB, OCB, EPL, UMU, UAE, NH2, GBM, AMB and AAP were given to them, the ten samples were representative of all types in which there were powdered type, emulsion type and suspension type of herbal medicine. Some of the herbal medicine had NAFDAC registration number others do not have NAFDAC registration number. Some of the plant parts used in preparing some of the herbal medicine such as leaves of *Heinsia crinata* and fruits of *Elais guineensis* was bought from Calabar main market, Watt, using its local vernacular names (Atama and Eyop respectively) and based on the researchers familiarity as a flavouring spice in soup preparation and was further authenticated in the Department of Botany, Biological Sciences, University of Calabar, Calabar, Nigeria. Test bacterial pathogens were sourced for from the University of Calabar Teaching Hospital (UCTH). The organisms were further identified and confirmed using standard protocols for cultural and morphological identification, as well as biochemical characterization of isolates.

Macroscopic Examination

The colour, odour, visibility, taste and other macroscopic examination was carried out on the collected herbal medicine.

Microbiological Evaluation

The sterility of the preparations - that is the herbal medicines was evaluated before the commencement of the phytochemical and antibacterial evaluation by plating it out on blood agar (BA), Cystine Lactose Electrolyte Deficient (CLED) agar, Sabourand Dextrose agar (SDA) and nutrient agar (NA) plates and incubated with $C0_2$ at 37°C for 24 hrs.for BA, without CO_2 for the other media for 24hrs and 72hrs for the SDA plates.

Preparation of Media
All the media used for the researched which were nutrient agar, CLED agar, Saboraud Dextrose Agar (SDA), TSI agar, Klinger Iron Agar (KIA), nutrient broth, blood agar (BA), muller hinton agar etc. were prepared according to the manufacturer's instructions.

Phytochemical Screening of Herbal Medicines
The herbal medicines were screened qualitatively while the herbal medicine with the code AAP was screened both qualitatively and quantitatively for the presence of tannin, alkaloids, cyanogenic glycosides, saponins, flavonoids, steroids, terpenoids, phlobatanins, reducing sugars and anthraquinones, using standard methods [21] [9][11] [6].

Preparation of Sensitivity Discs
Discs of about 6mm in diameter were made from Whatman's No.l filter paper using a paper puncher. Batches of 100 discs was transferred into Bijou bottle and sterilized in an oven at 140°C for 60 minutes.

Reconstitution of Herbal Medicines
Different concentrations of each of the herbal medicine were prepared by reconstituting different volumes of the herbal medicine in minimum volume of sterile distilled water. Required volume of herbal medicine was mixed in required volume of distilled water and reconstituted to a uniform viscous mixture using a glass rod to have the neat, 250mgml^{-1}, 125mgml^{-1} and 62.5mgml^{-1}. The sterile prepared discs were then counted into the mixture. More discs were added until the herbal medicine was completely used up. The approximate concentration of the herbal medicine absorbed by each discs was calculated using the formula below:

CE/N =CpD

Where:
CE = Concentration of reconstituted herbal medicine
N = Number of discs used
CpD = Concentration per disc.

Preparation of Inocula
From the stock cultures, which was maintained on nutrient agar slants at 4°C, colonies were transferred into nutrient broth using sterile inoculating wire-loop and incubated at 37°C for 24hrs, for each test bacteria.

Standardization of Inocula
The over-night broth cultures was diluted appropriately by gradually adding normal saline to it and the density of the inocula standardized by comparison with 0.5 McFarland standard of Barium sulphate solution [7], which is approximately 1.0 x 10^{6} cfuml^{-1} [2].

Antimicrobial Screening of Herbal Medicines
The antimicrobial sensitivity testing of the herbal medicines was performed using disc diffusion method on Mueller-Hinton agar [4]. The medium was prepared, sterilized, poured into plates and allowed to set. 0.10ml cultures of each test bacteria from the standardized inoculants was then inoculated onto the Mueller-Hinton agar plates aseptically and ensured that it was evenly distributed. Then using a sterile forceps, discs impregnated with different concentrations of the herbal medicines, was placed on each plate. The plates were allowed to stand for 30 Minutes at room temperature for effective diffusion before incubation at 37°C for 24hrs [5]. After incubation, zones of inhibition (diameter) formed on the medium was measured with a transparent meter rule and expressed in millimetre [19] to determine the antimicrobial effectiveness of the different concentrations of the herbal medicines [5].

Minimum Inhibitory Concentration (MIC)
MIC is defined as the lowest concentration where no visible turbidity is observed in the tube (bacteriostatic concentration). The minimum inhibitory concentration of the herbal medicines on test bacteria was carried out using tube dilution technique as described by Akinyemi *et al.,* (2005). Standardized suspension of the test bacteria was inoculated into a series of tubes of nutrient broth containing different concentrations of the herbal medicines and incubated at 37°C for 24hrs. MIC was then read after incubation as the least concentration of the herbal medicines that inhibited the growth of the test bacteria using turbidity as an index.

Minimum Bactericidal Concentration (MBC)
The MBC is defined as the lowest concentration where no bacterial growth is observed. This was determined by first selecting tubes that showed no growth during MIC determination, a loopful from each tube was then sub-cultured onto agar plates and incubated at 37°C for 24hrs [8]. The least concentration of the Minimum Inhibitory

Concentration (MIC) test at which no growth in the sub-cultured plates was observed, and noted as the minimum bactericidal concentration (MBC).

Statistical Analysis

Data obtained from measurement of zones of inhibition of each herbal medicines on test organisms was expressed as mean ± SE of triplicates. Two-way ANOVA factorial experiment, using Complete Randomized Design (CRD) design was used to analyze the effect of different solvents concentrations on antimicrobial activity of the test sample.

Table 1: Macroscopically Description of the Selected Herbal Medicine Samples

Sample Code	Description	NAFDAC Registration Number
DAH	A thick black oily emulsion with bad smell.	No
CNB	Foul odour dirty green suspension with particles.	No
OCB	Black solution, sweet but with little pungent smell.	Yes
EPL	Ginger-like smell, brown suspension with dirty brown subnatant.	Yes
UMU	Pungent smelling brown suspension.	No
UAE	Pungent smelling greenish brown suspension.	No
NH2	Sweet smelling dark coloured suspension	Yes
GBM	Sweet smelling clear pinkish solution	Yes
AMB	Sweet smelling, dark coloured solution.	Yes
AAP	Dried grinded powder made of plant parts (possibly leaves or roots) with a chocking smell on inhalation.	No

N/B: Sample AAP + hot water ⟶ Foul smelling greenish suspension.

Table 2: Enumerated Uses and Dosages of the Herbal Medicines by its Manufacturers

Sample Code	Expected Effects	Dosage
DAH	Enteric discomfort	One teaspoon into a teacup containing hot water.
CNB	Neisseria gonorrhea and other sexually transmitted infections.	One shot twice a day.
OCB	i. Detoxification of blood for optimal health and vibrant energy. ii. Prevention of kidney and blood infections. iii. Normalization of the operation of the intestine.	Adult, 3 table spoon daily. Children, 1 teaspoonful after meal preferable at night.
	Effective for the treatment of Staphylococcus aureus, urinary tract infection, thyphoid, cough-strept, Asthma bronchitis, enteric, Candida albican.	Adult, 2 shots twice daily. Adolescent 10-17yrs, one shot twice daily. Children 5-9yrs, two tablespoonfuls twice daily.
UMU	Internal heat and other feverish relieve.	Enema, early in the morning.
UAE	Measles and other infections.	Enema, early in the morning.
NH2	Candidiasis and other infections.	Adult, 10ml spoonful twice a day. Children 8-15yrs, 5ml spoonful twice a day.
GBM	Children and infant fevers, pain, windy gripes, relieve flatulence and restlessness; promptly render teething period perfectly mild.	3 months – 1yr, ½ teaspoon – 1 teaspoon 3-4 times daily. 1-5yrs, 1-2 teaspoon 3-4 times daily. 6-12yrs, 2-4 teaspoon 3-4 times daily.
AMB	Reduces free radical damage and removal of unwanted toxin in the body thereby aiding the immune system and the body's ability to resist diseases and for any infections: example, Staphylococcal infection.	Adult, 2tablespoonful (20ml) twice daily, morning and night. Children, 1teaspoon daily or as directed by physician.
AAP	All kinds of infections, example, Staphylococcal scalded skin syndrome	One shot twice daily.

RESULTS AND DISCUSSION

Ten (10) herbal medicines collected by purposive random sampling were analyzed for their microbial contaminants, phytochemical constituents and antibacterial activities. One (10%) of the analyzed samples was powder, one (10%) emulsion and the remaining eight samples (80%) were liquids (Table 1).

Blood detoxifier, reduction of free radical damage and removal of unwanted toxin in the body thereby aiding the immune system and the body's ability to resist disease formed 20% of the herbal medicine products in the Calabar herbal market, (They were OCB and EPL), 40% of the herbal medicine analyzed were claimed to be effective for enteric discomfort (which were DAH herbal remedy, OCB, UMU and UAE), 10% of the herbal medicine sampled,

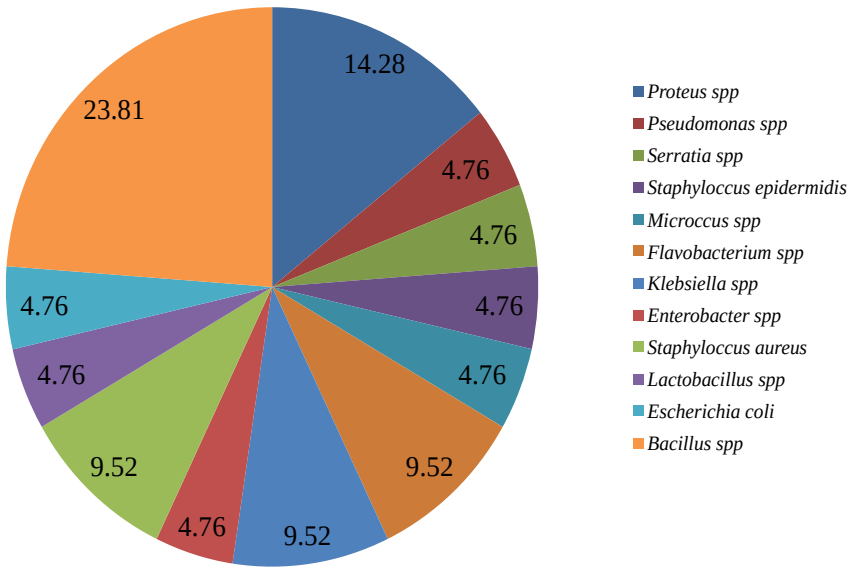
apart from been effective for enteric discomfort has been claimed by the locale to be effective for the treatment of measles popularly known as “atayayak” (which was UAE).

Twenty per cent (20%) of the herbal medicines are said to be effective against candidiasis (they were EPL and NH2 herbal remedy), 20% of the analyzed herbal medicine were claimed to be effective against sexually transmitted infections (which were CNB and NH2 herbal remedy). 10% effective against internal heat (which was UMU), while the rest (40%) of the herbal medicines sampled were claimed to manage urinary tract infection, asthma, bronchitis, infant fever and staphylococcal scalded skin syndromes (they were EPL, GBM, AMB and AAP) (Table 2).

During the study, a total of twelve (12) bacteria were isolated from the selected herbal medicines sampled. *Proteus* species was isolated from three of the herbal medicines – which were CNB, EPL and AAP, giving a frequency of occurrence of 14.28%. *Pseudomonas spp*. was isolated from one of the herbal medicines, which was CNB giving a frequency of occurence of 4.76%. *Serratia spp.* was equally isolated from one of the herbal medicine – which was CNB, giving a frequency of occurence of 4.76%.

Staphylococcus epidermidis was found in one of the herbal medicine – CNB, giving a frequency of occurence of 4.76%. *Micrococcus spp*. was found in one of the herbal medicine and was isolated from OCB, giving a frequency of occurence of 4.76%. *Bacillus spp*. was found in five (5) herebal medicines; they were isolated from OCB, EPL, NH2, GBM and AMB, giving a frequency of occurence of 23.81%. *Flavobacterium spp*. was isolated from OCB and UMU, giving a frequency of occurence of 9.52%. *Klebsiella spp*. was isolated from EPL and GBM, giving a frequency of occurence of 9.52%. *Enterobacter spp*. was found in one of the herbal medicines, it was isolated from UMU, giving a frequency of occurence of 4.67%, in the same vein, *Staphylococcus aureus* was isolated from two (2) of the herbal medicine, which were UMU and UAE, giving a frequency of occurence of 9.52%. *Lactobacillus spp*. was isolated from one of the herbal medicines – UAE, giving a frequency of occurence of 4.76% and *Escherichia coli* was equally isolated from this same UAE herbal medicine, giving a frequency of occurence of 4.76%. A pie chart depicting the frequency of occurence of bacteria isolated from the selected herbal medicine is presented in figure 1 as in the table 3.

Figure 1 Frequency of bacteria isolated from the selected herbal medicines



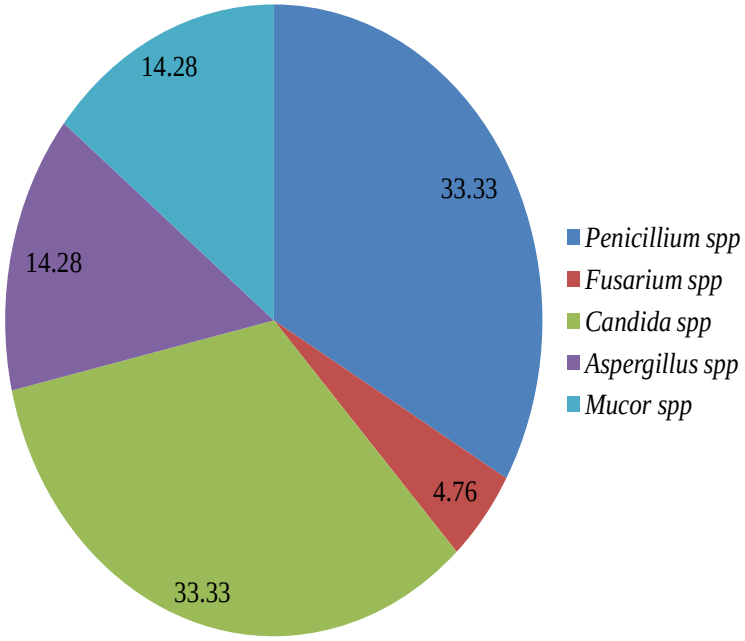


Figure 2: Frequency of fungi isolated from the selected herbal medicine

A total of five (5) different fungi were equally isolated from the selected herbal medicines sampled. *Penicillum spp*. was isolated in seven (7) of the herbal medicines, they were DAH herbal remedy, CNB, OCB, NH2 herbal remedy, GBM, AMB and AAP, giving a frequency of occurence of 33.33%.

Similarly, *Fusarium spp*. was found in one of the herbal medicines and it was isolated from DAH herbal remedy, giving a frequency of occurence of 4.76%. *Candida spp.* was found in seven (7) of the herbal medicines, which were DAH herbal remedy, CNB, EPL, UMU, UAE, AMB and AAP, giving a frequency of occurence of 33.33%. *Aspergillus spp*. was found in three (3) of the herbal medicines; it was isolated from CNB, UAE and AAP, giving a frequency of occurence of 14.28%. The last but not the least fungi isolated was *Mucor spp*., it was isolated from three (3) of the herbal medicines which were CNB, EPL and UMU, giving a frequency of occurence of 14.28%. A pie chart depicting the frequency of fungi isolated from the selected herbal medicine is presented in figure 2 as in the table in table 4.

The result of the phytochemical screening of the selected herbal medicines sold in Calabar (table 5) shows reducing compounds and polyphenol to be present in much excess amount (+ + +) in 50% and 30% of the herbal medicines respectively while, alkaloids were present in excess amount (+ +) in 70% of the herbal medicines. Cardiac glycosides were present (+) in 90% of the herbal medicines sampled, while saponins was absent (-) in 80% of the herbal medicines sampled. Tannin was present in 60% of the selected herbal medicine. Phlobatanins, triperpenes, anthraquinones and hydroxylmethyl anthraquinones were absent (-) in 80%, 70%, 90%, and 80% respectively of the herbal medicine sampled.

DAH herbal remedy contained polyphenol present in much excess (+ + +), alkaloids and tannins present in excess (+ +), phlobatanins present (+) while cardiac glycosides, saponins, flavonoids, reducing compounds, triterpenes, anthraquinones and hydroxylmethyl anthraquinones were absent (-). In CNB (herbal) medicine, reducing compounds was present in much excess (+ + +), polyphenol was present in excess (+ +), alkaloids, cardiac glycosides, tannins and flavonoids were present (+) while saponins, phlobatanins, triterpenes, anthraquinones and hydroxymethyl anthraquinones were absent (-).

Table 5: Summary of the phytochemical composition of selected herbal medicines sold in Calabar

S/No	Chemical Constituents	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 10 (Ethanolic Extract)
1.	Alkaloids	++	+	+	++	++	++	++	+++	++	++	+
2.	Cardiac Glycosides	-	+	+	+	+	+	+	+	+	+	+
3.	Saponins	-	-	-	+	-	-	+	-	-	-	-
4	Tannins	++	+	-	+	-	+	+	-	-	+	+
5	Flavonoids	-	+	+	+	-	-	+	-	-	+	+
6.	Reducing Compounds	-	+++	+++	+++	+	++	++	++	+++	+++	++
7.	Polyphenol	+++	++	-	++	++	++	++	++	++	+++	+++
8.	Phlobatanins	+	-	-	-	-	-	+	-	-	-	-
9.	Triterpenes	-	-	-	-	-	+	-	+	+	-	-
10.	Anthraquinones	-	-	-	-	+	-	-	-	-	-	-
11.	Hydroxymethyl anthraquinones	-	-	++	+	-	-	-	-	-	-	-

KEY

+	PRESENT	SAMPLE	1 --- DAH HERBAL REMEDY	SAMPLE	6 --- UAE
++	PRESENT IN EXECESS	"	2 --- CNB	"	7 --- NH2 HERBAL REMEDY
+++	PRESENT IN MUCH EXCESS	"	3 --- OCB	"	8 --- GBM
	ABSENT	"	4 --- EPL	"	9 --- AMB
-		"	5 --- UMU	"	10 --- AAP

Table 3: Morphological and Biochemical Characteristics of Bacteria Isolates

ISOLATE CODE	GRM RXN	SHAPE	CATA-LASE	OXIDASE	CITRATE	INDOLE	MOTILITY	VOGUS PRO-SKAUER	METHYL RED	UREASE	ORTHNI-THINE DECARBOX-YLASE	FERMENTATION OF SUGARS				KIA MEDIUM				COAGUL ASE	SUSPECTED ORGANISM
												LAC	MAN	GLU	SUC	SLOPE	BUTT	H_2S	GAS		
1	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	-	-	-
2A	-ve	Coco Bacilli	+	-	-	+	+	-	+	+	-	-	-	+	+	R	Y	+	+	-	*Proteus spp.*
2B	-ve	Rods	+	+	+	-	+	-	+			-	+	+		R	R	-	-	-	*Pseudomonas spp.*
2C	-ve	Rods	+	-	+	-	+	+	-	-	+	+	+	+	+	Y	Y	-	-	-	*Serratia spp.*
2D	+ve	cocci	+	-	-	-	-	-	+	+		+	-	+	+						*Staphylococcus epidermidis*
3A	+ve	Cocci	+	+	-	-	-	-	+	+		-	-	-	-					-	*Micrococcus spp.*
3B	+ve	Rods	+	-	-	-	+	-	+	+		+	+	+	+					-	*Bacillus spp.*
3C	-ve	Rods	+	+		-	-			-		-	-	+	-				-	-	*Flavobacterium spp.*
4A	-ve	Rods	+	-	+	-	-	+	-	+		+	+	+	+					-	*Klebsiella spp.*
4B	-ve	Coco bacilli	+	-	-	+	+	-	+	+	-	-	-	+	+	Y	Y	-	+	+	*Proteus spp.*
4C	+ve	Rods	+	-	-	-	+	-	+	+	-	+	+	+	+	R	R	+	+	-	*Bacillus spp.*
5A	-ve	Rods	+	-		-	+			-		+	+	+	+				+	-	*Enterobacter spp.*
5B	+ve	Cocci	+	-	+	-	-	+	-	+	+	+	+	+	+	Y	Y	-		-	*Staphylococcus aureus*
5C	-ve	Rods	+	+	-	-	-	-	+	-	+	-	-	+	-				-	+	*Flavobacterium spp.*
6A	+ve	Rods	-				-						-	+							*Lactobacillus spp.*
6B	-ve	Rods	+	-	-	+	+	-	+	-	+	+	+	+	+	Y	Y	-	+	-	*Escherichia coli*
6C	+ve	Cocci in cluster		-								+	+	+	+						*Staphylococus aureus*
7	+ve	Rods	+	-	-	-	+	-	+	+		+	+	+	+					-	*Bacillus spp.*
8A	+ve	Rods	+	-	-	-	+	-	+	+		+	+	+	+					-	*Bacillus spp.*
8B	-ve	Rods	+	-	+	-	-	+	-	+	-	+	+	+	+			-	+		*Klebsiella spp.*
9	+ve	Rods	+	-	-	-	+	-	+	+		+	+	+	+					-	*Bacillus spp.*
10	-ve	Coco bacilli	+	-	-	+	+	-	+	+	-	-	-	+	+	R	Y	+	+		*Proteus spp.*

KEY

Y = Acid reaction
R = Red-pink (alkaline reaction)
+ = Positive
- = Negative
LAC = Lactose
GLU = Glucose
SUC = Sucrose

Table 4: Morphology and Identification of Fungal Isolates

ISOLATE CODE	COLOUR OF AERIAL HYPHAE	COLOUR OF SUBSTRATE HYPHAE	NATURE OF HYPHAE	SPORE SHAPE	POSSIBLE ORGANISM
1A	Green	Brown	Septate	Round Sporangium	*Penicillium spp.*
1B	Pink	Dark brown	Septate	Green concave shape conidia	*Fusarium spp.*
1C	Creamy		Septate	Oval condidia	Yeast
2A	Yellow	Brown	Septate	Round sporangium	*Aspergillus spp.*
2B	Dark	White	Aseptate		*Mucor spp.*
2C	Creamy		Septate	Round Sporangium	Yeast
2D	Green	Brown	Septate		*Penicillium spp.*
3	Green	Brown	Septate	Round Sporangium	*Penicillium spp.*
4A	Creamy		Septate	Round Sporangium	Yeast
4B	Dark	White	Aseptate	Round Sporangium	*Mucor spp.*
5A	Dark	White	Aseptate	Round Sporangium	*Mucor spp.*
5B	Creamy		Septate		Yeast
6A	Yellow	Brown	Septate	Oval conidia	*Aspergillus spp.*
6B	Creamy		Septate		Yeast
7	Green	Brown	Septate	Round Sporangium	*Penicillium spp.*
8	Green	Brown	Septate	Round Sporangium	*Penicillium spp.*
9A	Creamy		Septate	Round Sporangium	Yeast
9B	Green	Brown	Septate		*Penicillium spp.*
10A	Yellow	Brown	Septate	Oval conidia	*Aspergillus spp.*
10B	Green	Brown	Septate	Round Sporangium	*Penicillium spp.*
10C	Creamy		Septate		Yeast

OCB contained reducing compounds present in much excess (+ + +), hydroxylmethyl anthraquinones present in excess (+ +), cardiac glycosides, alkaloids and flavonoids present (+) while saponins, tannins, polyphenol, phlobatanins, triterpenes and anthraquinones were absent (-). In EPL, reducing compounds were present in much excess (+ + +), polyphenol and alkaloids were present (+ +), cardiac glycosides, saponins, tannins, flavonoids and hydroxymethyl anthraquinones were present (+) while phlobatanins, triterpenes and anthraquinones were absent (-). In UMU, alkaloids and polypphenol were present in excess (+ +), cardiac glycosides, anthraquinones and reducing compounds were present (+) while saponins, tannins, flavonoids, phlobatanins, triterpenes and hydroxymethyl anthraquinones were absent (-). UAE was composed of alkaloids, reducing compounds and polyphenol present in excess (+ +), cardiac glycosides, tannins and triterpenes present (+) while saponins, flavonoids, phlobatanins, anthraquinones and hydroxymethyl anthraquinones were absent (-).

Similarly, NH2 herbal remedy was composed of alkaloids, reducing compounds and polyphenol present in excess (+ +), cardiac glycosides, saponins, tannins, flavonoids and phlobatanins were present (+) while triterpenes, anthraquinones and hydroxymethyl anthraquinones were absent (-). GBM had alkaloids present in much excess (+ + +), reducing compounds and polyphenol were present in excess (+ +), cardiac glycosides and triterpenes were present (+) while saponins, tannins, flavonoids, phlobatanins, anthraquinones and hydroxhmethyl anthraquinones were absent (-).

In addition, AMB were composed of reducing compounds present in much excess (+ + +), alkaloids and polyphenol present in excess (+ +), cardiac glycosides and triterpenes present (+) while saponins, tannins, flavonoids, phlobatanins, anthraquinones and hyroxymethyl anthraquinones were absent (-). AAP contained reducing compounds and polyphenol present in much excess (+ + +), alkaloids present in excess (+ +), cardiac glycosides, tannins and flavonoids present (+) while saponins, phlobatanins, triterpenes, anthraquinones and hydroxymethyl anthraquinones were absent (-). The ethanolic extract of the AAP gave quite a different phytochemical composition result from the aqueous extracts. It had reducing compounds present in excess (+ +) whereas, the aqueous extract had it present in much excess (+ + +) also, alkaloids were present (+) whereas the aqueous extract had it present in excess (+ +). All other phytoconstituents of the AAP were the same as that of the aqueous extract.

The ten herbal medicines sampled for the research were powdered type, emulsion type and liquid type. The liquid and emulsion type were aqueous made, and as such, their phytochemical screening was carried out directly without any additional procedure. That of AAP was carried out using its aqueous extract and ethanolic extract after which quantitative determination of selected phytochemicals was determined for it.

Flavonoids made up 18.40% of AAP phytoconstituents, alkaloids 10.00% while reducing compounds made up 15.97% of it. Glycosides made up 0.90% while tannins made up 0.52% of it. The data obtained, through the quantitative determination of phytochemical in AAP is presented in table 6.

Table 6: Percentage Composition of Crude Alkaloids, Tannins, Glycosides, Flavonoids and Reducing Sugar in AAP

Name of Sample	Alkaloids %	Tannins %	Glycosides %	Flavonoids %	Reducing Sugar %
AAP	10.00	0.52	0.90	18.40	15.97

Antibacterial Activity of the Selected Herbal Medicines

The antimicrobial sensitivity testing of the herbal medicines was carried out using disc diffusion method on Muller-Hinton agar. Different concentrations of each of the herbal medicines were prepared, which were 500mgml^{-1}, 250mgml^{-1}, 125mgml^{-1} and 62.5mgml^{-1}, of which zones of inhibition were measured and expressed as its mean $\pm$ standard error. There were significant differences ($P < 0.05$) between the various herbal medicines used for the study. The herbal medicines DAH herbal remedy, CNB, OCB, EPL, UMU, UAE, NH2 herbal remedy, GBM, AMB and AAP has the following mean zone of inhibition against *Staphylococcus aureus* 20.88 ± 3.60, 0.00 $\pm$ 0.00, 9.63$\pm$ 0.88, 0.00 $\pm$ 0.00, 8.00 $\pm$ 0.85, 2.63 $\pm$ 0.25, 8.75 $\pm$ 0.98, 9.38 $\pm$ 1.06, 9.63 $\pm$ 1.06 and 0.00 $\pm$ 0.00 millimetres respectively.

The significant difference ($P < 0.05$) between the herbal medicines DAH herbal remedy, CNB, OCB, EPL, UMU, UAE, NH2 herbal remedy, GBM, AMB and AAP were 11.00 $\pm$ 2.48, 3.88 $\pm$ 0.34, 5.63 $\pm$ 0.43, 0.00 $\pm$ 0.00, 0.00 $\pm$ 0.00, 5.75 $\pm$ 0.36, 5.38 $\pm$ 0.35, 5.38 $\pm$ 0.41, 5.50 $\pm$ 0.43 and 0.00 $\pm$ 0.00 millimetres respectively against *Klebsiella pneumonia*. For *Streptococcus, faecalis*, there were no significant difference between the effect of herbal medicine CNB, OCB, EPL, UMU, UAE, NH2 herbal remedy, AMB, and AAP on the tested microorganism, but there was significant difference in the effect of DAH herbal remedy and GBM on the clinical isolates.

The effects of DAH herbal remedy, CNB, OCB, EPL, UMU, UAE, NH2 herbal remedy, GBM, AMB and AAP on *Pseudomonas aeroginosa* were: 13.63 $\pm$ 2.18, 0.00 $\pm$ 0.00, 3.38 $\pm$ 0.34, 5.88 $\pm$ 0.41, 0.00 $\pm$ 0.00, 1.88 $\pm$ 0.25, 1.75 $\pm$ 0.50, 1.88 $\pm$ 0.75, 5.13 $\pm$ 0.35 and 0.00 $\pm$ 0.00 millimetres respectively. For *Micrococcus spp.*, the effect of the 10 herbal medicines - DAH herbal remedy, CNB, OCB, EPL, UMU, UAE, NH2 herbal remedy, GBM, AMB and AAP were 9.50 $\pm$ 2.83, 0.00 $\pm$ 0.00, 0.00 $\pm$ 0.00, 0.00 $\pm$ 0.00, 0.00 $\pm$ 0.00, 0.00 $\pm$ 0.00, 0.00 $\pm$ 0.00, 0.00 $\pm$ 0.00, 0.00 $\pm$ 0.00 and 0.00 $\pm$ 0.00 millimetres respectively.

That of *Proteus vulgaris* was: 10.63 $\pm$ 1.42, 0.00 $\pm$ 0.00, 6.75 $\pm$ 0.71, 0.00 $\pm$ 0.00, 0.00 $\pm$ 0.00, 0.00 $\pm$ 0.00, 0.00 $\pm$ 0.00, 8.88 $\pm$ 1.44, 3.75 $\pm$ 2.35, and 0.00 $\pm$ 0.00 millimetres respectively. In the case of *Salmonella typhii*, the antibacterial activity profile of the selected herbal medicine: DAH herbal remedy, CNB, OCB, EPL, UMU, UAE, NH2 herbal remedy, GBM, AMB and AAP to it were: 15.63 $\pm$ 2.80, 0.00 $\pm$ 0.00, 8.50 $\pm$ 0.65, 0.00 $\pm$ 0.00, 0.00 $\pm$ 0.00, 2.00 $\pm$ 0.50, 8.25 $\pm$ 0.65, 8.00 $\pm$ 0.73, 8.63 $\pm$ 1.12 and 0.00 $\pm$ 0.00 millimetres respectively. With *Escherichia coli*, they were: 12.50 $\pm$ 1.09, 0.00 $\pm$ 0.00, 4.00 $\pm$ 0.41, 5.5 $\pm$ 0.43, 5.75 $\pm$ 0.43, 0.00 $\pm$ 0.00, 3.88 $\pm$0.44, 0.00 $\pm$ 0.00, 3.75 $\pm$ 0.45 and 0.00 $\pm$ 0.00 millimetres respectively. Table 7 shows the zones of inhibition of the growth of the tested clinical bacteria using selected herbal medicines. According to table 8, some of the herbal medicines evaluated showed the same or almost the same zones of inhibition as that of orthodox antibiotics. Meaning that they can be effectively used for the treatment of diseases caused by these test bacteria if only the manufacturers of these herbal medicines will follow Good Manufacturing Practice (GMP) in order to bring the contaminants to its permissibly limit.

Table 7: Susceptibility of Test Bacteria to Selected Herbal Medicines

Test Bacteria	Herbal Medicine Sample Code Number 1	2	3	4	5	6	7	8	9	10
Staphylococcus aureus	20.88 ± 3.0^a	0.00 ± 0.00^d	9.63±0.88^b	0.00±0.00^d	8.00±0.85^b	2.63±0.25^c	8.75±0.98^b	9.38±1.06^b	9.63±1.01^b	0.00±0.00^d
Klebsiella pneumonia	11.00±2.48^a	3.88±0.34^c	5.63±0.43^b	0.00±0.00^d	0.00±0.00^d	5.75±0.36^b	5.38±0.35^b	5.38±0.41^b	5.50±0.43^b	0.00±0.00^d
Streptococcus faecalis	17.75±1.55^a	0.00±0.00^c	0.00 ±0.00^c	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c	11.25±1.18^b	0.00±0.00^c	0.00±0.00^c
Pseudomonas aeroginosa	13.63±2.18^a	0.00±0.00^d	3.38±0.34^c	5.88±0.34^c	0.00±0.00^d	1.88±0.25^c	1.75±0.50^c	1.88±0.75^c	5.13±0.35^b	0.00±0.00^d
Micrococcus spp.	9.50±2.83^a	0.00±0.00^b	0.00±0.00^b	0.00±0.00^b	0.00±0.00^b	0.00±00^b	0.00±00^b	0.00±0.00^b	0.00±0.00^b	0.00±0.00^b
Proteus vulgaris	10.63±1.42^a	0.00±0.00^d	6.75±0.71^b	0.00±0.00^d	0.00±0.00^d	0.00±0.00^d	0.00±0.00^d	8.88±1.44^b	3.75±2.35^c	0.00±0.00^d
Salmonella typhii	15.63±2.80^a	0.00±0.00^d	8.50±0.65^b	0.00±0.00^d	0.00±0.00^d	2.00±0.50^c	8.25±0.65^c	8.00±0.73^b	8.63±1.12^b	0.00±0.00d
Escherichia coli	12.50±1.09^a	0.00±0.00^d	4.00±0.41^b	5.50±0.43	5.75±0.43^b	0.00±0.00^d	3.88±0.44^c	0.00±0.00^d	3.75±0.45^c	0.00±0.00^d

Mean (average) zone of inhibition with same lettered superscript signifies no significant difference while mean (average) zone of inhibition with different lettered superscript signifies that there was significant difference in the anti-bacterial effect of the herbal medicine to the test bacteria.

KEY

1 ----- DAH herbal remedies
1 ----- CNB
2 ----- OCB
4 ----- EPL
5 ----- UMU
6 ----- UAE
7 ----- NH2 herbal remedy
8 ----- GBM
9 ----- AMB
10 ----- AAP

Table 8:Antibacterial Activity of Selected Antibiotics (Control) to Test Bacteria

Test Bacteria	Mean Zones of Inhibition (mm) * +ve/-ve Controls			
	CN	CPX	SXT	W
Staphylococcus aureus	20 ± 1.4^a	18 ± 0.5^b	18 ± 0.8^b	NA
Streptococcus faecalis	19 ± 0.8^a	18 ± 2.0^b	17 ± 2.2^b	NA
Micrococcus spp	12±1.4^c	24±2.2^a	14±1.2^b	NA
P. aeruginosa	NA	27 ± 0.5^a	NA	NA
Escherichia coli	21 ± 1.2^b	24 ± 0.5^a	11 ± 0.5^c	NA
Samonella typhi	18 ± 0.5^b	27 ± 1.2^a	NA	NA
K. pneumonia	28 ± 0.5^a	25 ± 0.8	18 ± 1.2^c	NA
Proteus vulgaris	25 ± 7.2^a	23 ± 1.2^b	NA	NA

*Data are expressed as mean ±standard error (SE) of triplicate trials. Values with different superscript across the rows are statistically significant ($P < 0.05$). *CN (gentamycin 10 µg), CPX (ciprofloxacin 30 µg), SXT (septrin 30 µg) = positive controls and W (water) = negative control. NA = No activity.*

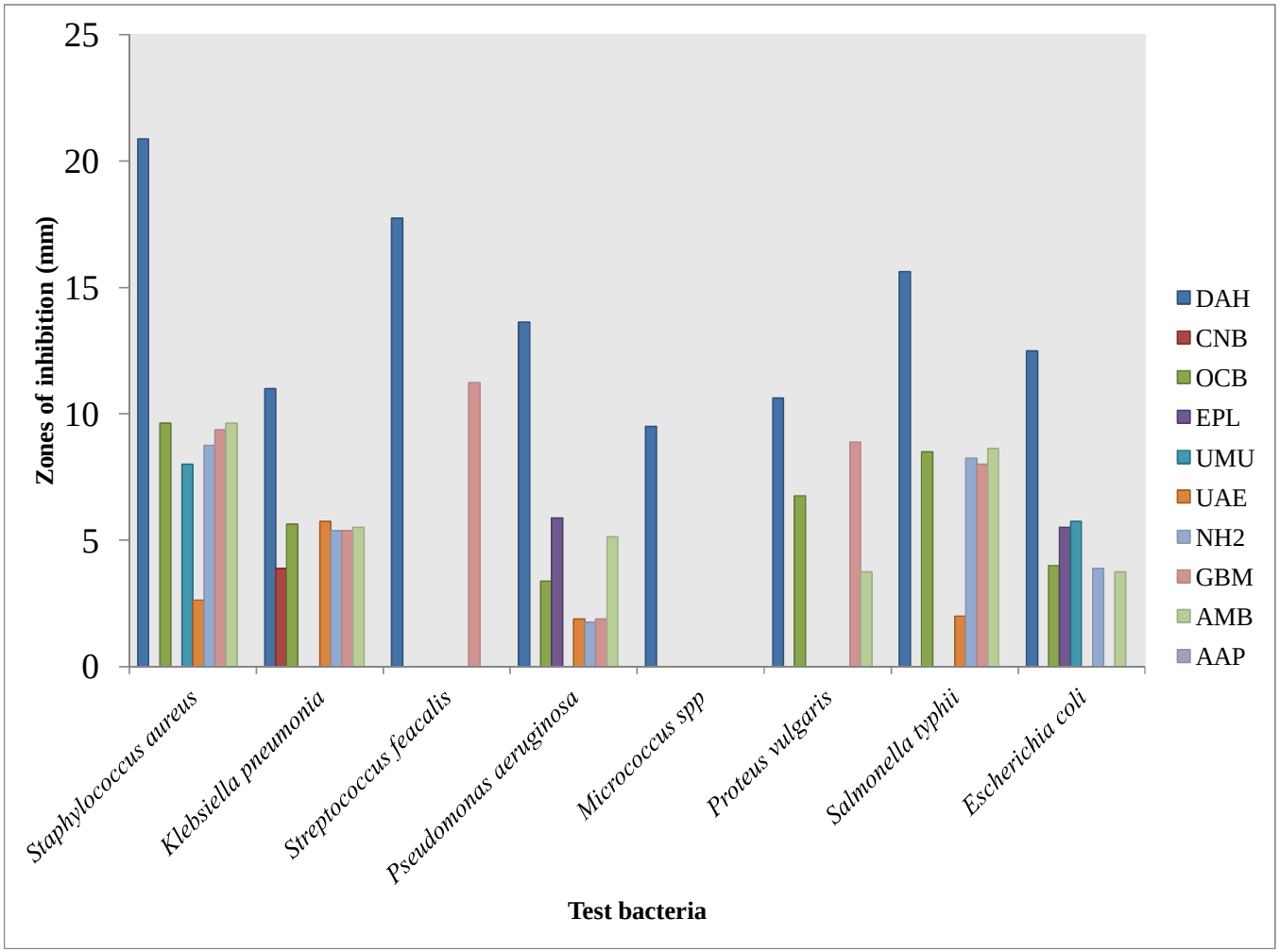


Figure 3: The antibacterial profile of selected herbal medicine to test bacteria

Table 9: Summary of the MIC and MBC Regimes of Evaluated Herbal Medicines to Test Bacteria

TEST BACTERIA	1 ($mgml^{-1}$)		2($mgml^{-1}$)		3($mgml^{-1}$)		4(mgm^{-1})		5($mgml^{-1}$)		6 ($mgml^{-1}$)		7($mgml^{-1}$)		8 ($mgml^{-1}$)		9(mgm^{-1})		10 ($mgml^{-1}$)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	125	250	≥1000	≥2000	125	≥500	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	250	250	125	500	≥1000	≥2000
Klebsiella pneumonia	62.5	≥125	≥1000	≥2000	250	≥500	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	<62.5	≤62.5	≥1000	≥2000
Micrococcus spp.	62.5	125	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	250	>500	125	250	≥1000	≥2000	125	250	≥1000	≥2000
Proteus vulgaris	≤62.5	500	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	250	>500	<62.5	>500	≥1000	≥2000	125	>500	≥1000	≥2000
Pseudomonas aeroginosa	<62.5	125	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	125	>500	≥1000	≥2000	125	>500	≥1000	≥2000
Salmonella typhii	<62.5	125	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	125	>500	500	500	≥1000	≥2000	<62.5	<62.5	250	>500
Staphylococcus aureus	62.5	125	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	≤62.5	125	≥1000	≥2000
Streptococcus faecalis	<62.5	125	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	≥1000	≥2000	≥62.5	250	≥1000	≥2000	<62.5	250	250	>500

KEY

MIC --- Minimum Inhibitory Concentration
MBC --- Minimum Bacteriocidal Concentration
$Mgml^{-1}$ ---Miligram per millilitre
Spp. ---Species

1 --- DAH herbal remedy
2 --- CNB
3 --- OCB
4 --- EPL
5 --- UMU
6 --- UAE
7 --- NH2 herbal remedy
8 --- GBM
9 --- AMB
10 --- AAP

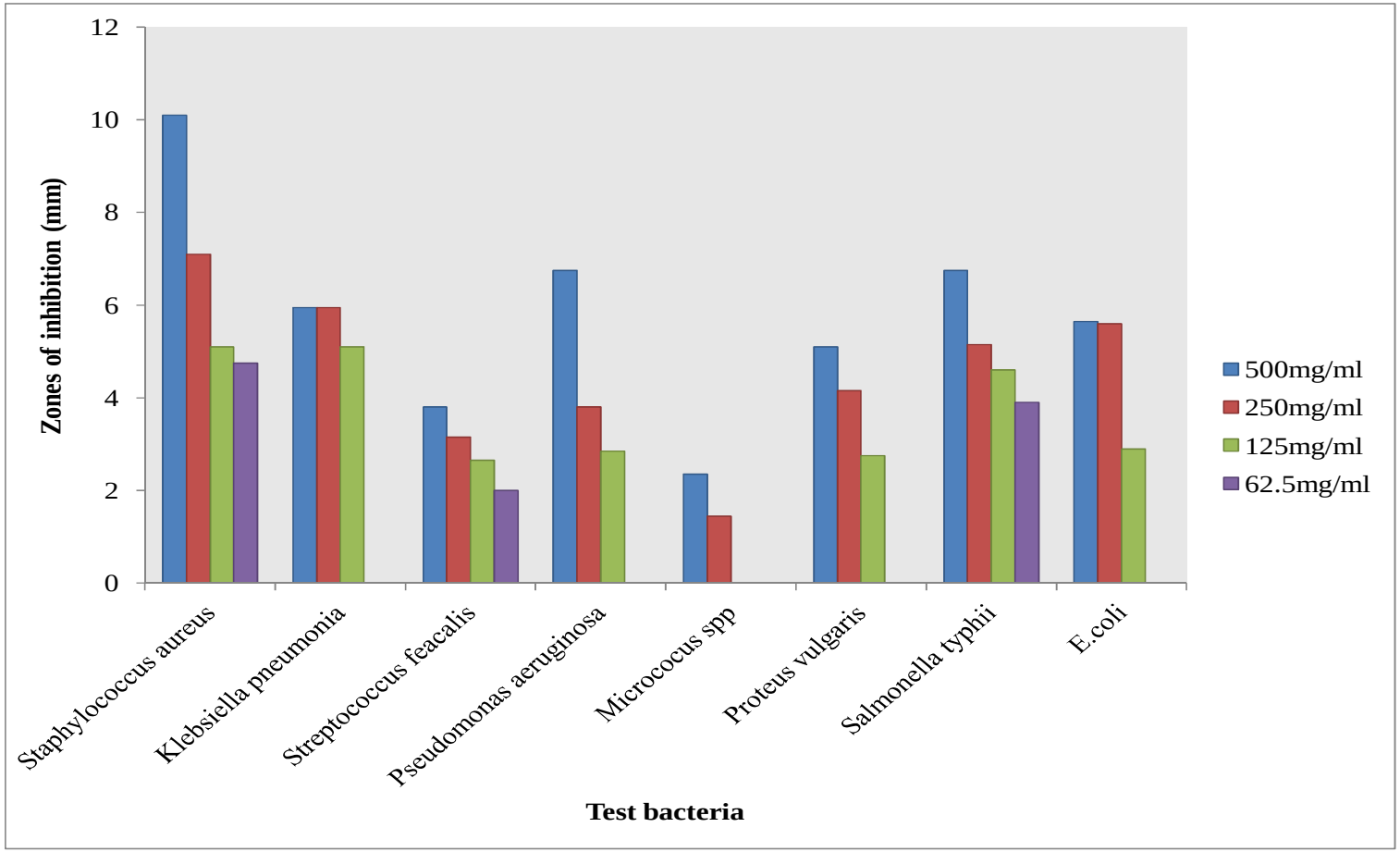


Figure 4: Effect of the herbal medicine concentrations on test bacteria

The minimum inhibitory concentration (MIC) of DAH herbal remedy for the different test bacteria ranged between 62.5 – 125 $mgml^{-1}$ that of CNB, EPL and UMU was ≥ 1000 $mgml^{-1}$. The herbal medicine OCB and UAE has its MIC between the range of 125 - ≥ 1000 $mgml^{-1}$, while that of NH2 herbal remedy ranged between < 62.5 - ≥ 100 $mgml^{-1}$. Also, the MIC of GBM and AAP ranged between 250 - ≥ 1000 $mgml^{-1}$ and that of AMB ranged between < 62.5 to 125$mgml^{-1}$.

The minimum bactericidal concentration activity (MBC) of the herbal medicines for different bacteria ranged between 125 – 250 $mgml^{-1}$ for DAH herbal remedy, ≥ 2000 $mgml^{-1}$ for CNB, EPL and UMU, >500 - ≥ 2000 $mgml^{-1}$ for OCB, UAE and AAP. Also, the MBC of NH2 herbal remedy and GBM ranged between 250 - ≥ 2000 $mgml^{-1}$ while, that of AMB ranged between < 62.5 – 125 $mgml^{-1}$ (Table 9).

Effect of Concentration of Herbal Medicine on Test Bacteria

All test clinical isolates were sensitive to the herbal medicines. Four varying concentration of the herbal medicines that is 500$mgml^{-1}$, 250$mgml^{-1}$, 125$mgml^{-1}$ and 62.5$mgml^{-1}$ formed the factor A used for the statistical analysis. Out of the four varying concentration of the herbal medicines, 500$mgml^{-1}$ concentration gave better results for all the test bacteria.

There were significant differences in the effect of the concentration of the herbal medicine on *Staphylococcus aureus* using the mean zone of inhibition ± S.E, which were 10.1 ± 2.81, 7.1 ± 1.74, 5.1 ± 1.11 and 4.75 ± 0.53 millimetres respectively. That of *Klebsiella pneumonia* was 5.95 ± 1.86, 5.95 ± 0.51, 5.1 ± 0.34 and 0.00 ± 0.00 millimetres for 500$mgml^{-1}$, 250$mgml^{-1}$, 125$mgml^{-1}$ and 62.5$mgml^{-1}$.

The concentration effect of the herbal medicine on *Streptococcus faecalis* gave a mean zone of inhibition of 3.8 ± 1.57, 3.15 ± 1.45, 2.65 ± 1.11 and 2.00 ± 1.07 millimetres while that of *Pseudomonas aeroginosa* were 6.75 ± 2.12, 3.80 ± 1.29, 2.85 ± 1.29 and 0.00 ± 0.00 millimetres and *Micrococcus spp*. 2.35 ± 1.12, 1.45 ± 0.67, 0.00 ± 0.00 and 0.00 ± 0.00 millimetres for 500$mgml^{-1}$, 250$mgml^{-1}$, 125$mgml^{-1}$ and 62.5$mgml^{-1}$.

The other test bacteria – *Proteus vulgaris***,** *Salmonella typhii* and *Escherichia coli* gave a mean zone of inhibition of 5.1 ± 1.49, 4.15 ± 1.00, 2.75 ± 0.99, 0.00 ± 0.00, 6.75 ± 2.24, 5.15 ± 1.43, 4.6 ± 0.81, 3.9 ± 0.49, 5.65 ± 1.54, 5.6 ± 1.19, 2.9 ± 1.01 and 0.00 ± 0.00 millimetres for 500$mgml^{-1}$, 250$mgml^{-1}$, 125$mgml^{-1}$ and 62.5$mgml^{-1}$ (Table 10).

TABLE 10: Effect of the Herbal Medicine Concentration on Test Bacteria

Test bacteria	Concentrations ($mgml^{-1}$)			
	500	250	125	62.5
Staphylococcus aureus	10.1 ±2.81^{a}	7.1 ± 1.74^{b}	5.1 ± 1.11^{c}	4.75 ± 0.53^{d}
Klebsiella pneumonia	5.95 ± 1.86^{a}	5.95 ± 0.51^{a}	5.1 ± 0.34^{b}	0.00 ± 0.00^{c}
Streptococcus faecalis	3.8 ± 1.57^{a}	3.15 ± 1.45^{b}	2.65 ± 1.11^{c}	2.00 ± 1.07^{d}
Pseudomonas aeroginosa	6.75 ± 2.12^{a}	3.8 ± 1.29^{b}	2.85 ± 1.29^{c}	0.00 ± 0.00^{d}
Micrococcus spp.	2.35 ± 1.12^{a}	1.45 ± 0.67^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}
Proteus vulgaris	5.1 ± 1.49^{a}	4.15 ± 1.00^{b}	2.75 ± 0.99^{c}	0.00 ± 0.00^{d}
Salmonella typhii	6.75 ± 2.24^{a}	5.15 ± 1.43^{b}	4.6 ± 0.81^{b}	3.9 ± 0.49^{b}
Escherichia coli	5.65 ± 1.54^{a}	5.6 ± 1.19^{a}	2.9 ± 1.01^{b}	0.00 ± 0.00^{c}

In the rows, mean zones of inhibition with same lettered superscript signifies that there was no significant difference in the effect of the concentrations on test bacteria. While mean zones of inhibitions with different lettered superscript signifies that there were significant difference in the effect of the concentrations on test bacteria.

Medicinal plant materials carry a large number of microbes originating from the soil. Micro-organisms of various kinds normally adhered to leaves, stems, flowers, seeds and roots. Additional contaminants may also be introduced during harvesting, handling and production of various herbal remedies since no conscious efforts are made to decontaminate the herbs other than washing them.

Phyto-pharmaceutical drugs or any orthodox pharmaceutical product to be used internally in the human or animal body should be sterile to avoid infecting the user of the product with the contaminant. According to figure 1 and 2, isolating bacteria and fungi respectively in such a frequency does not mean that they are normal flora of the plants used to prepare the medicines, they are the contaminants.

Table three and four shows the bacterial and fungal loads respectively of the sampled herbal medicines, there, one can see that UMU (herbal medicine) had a bacterial load of 27.7 ± 3.39 x 10^{9} $cfuml^{-1}$ followed by UAE with 24.5 ±

4.98 x 10^8 cfuml^{-1} which are on the high side, whereas the world health organization, British Pharmacopoeia and the United State Pharmacopoeia have recommended tolerable microbial limits in non-sterile pharmaceutical products which include 10^7 cfuml^{-1} bacteria and 10^5 cfuml^{-1} fungi. The other sampled herbal medicines were able to meet this limit to some extent. The possible reason why the microbial load of UMU and UAE were high, may be from their environment where they were harvested, method of preparation or they were been preserved in a contaminated vessel. Microbial contaminants of herbal medicine may also occur through handling by personnel who are infected with pathogenic bacteria during harvest/collection of the herb, post-harvest processing and the manufacturing process. This could be controlled by implementing best practice guidelines such as GACP and GMP [1].

Other reasons for the contamination of the herbal medicine could be that the water used in preparing or diluting the juice extract of the plant part used for the medicine contained the contaminating bacteria, or if the final product-the suspension of the solution of the herbal medicine was stored in improperly washed containers [20], as in CNB in which the seller has to fetch rain water to rinse the container before dispensing the herbal medicine to the researcher who pretended to be down with an ailment.

The World Health Organisation survey indicated that about 70-80% of the world population particularly in developing countries like Nigeria rely on non-conventional medicines mainly of herbal origins for their primary health care [24][1]. This is because herbal medicines are accessible and cheap. Therefore the quality and safety of herbal preparations are also of great concern. The WHO (1993) explained that quality is the basis of reproducible, efficacy and safety of herbal drugs, and to ensure the standard of research on herbal medicines, the quality of the plant materials or preparation is of utmost importance.

The phytochemical studies of selected herbal medicines sold in Calabar showed the presence of alkaloids, glycosides, reducing compound in about 70% of the herbal medicines as been present in excess, present and present in much excess respectively. Saponin was found in 20% of the herbal medicine. Tanins and flavonoids were found in 40% of the herbal medicine while polyphenol was found in 80% of the herbal medicine. Phlobatanins, triterpenes, anthraquinones and hydroxymethyl anthraquinones were found in 20%, 30%, 10% and 20% of the herbal medicine studied respectively.

In this study, DAH herbal remedy showed greater zones of inhibition to all the clinical bacteria with zones of inhibition ranging from 9.5-20.88mm across the column, meaning that DAH herbal remedy had broad spectrum of activity to both gram positive and gram negative bacteria. The phytochemical constituent of such herbal medicine could be research into further in other to use it in combating the ever increasing multidrug resistance of bacteria to antibiotic. This finding is similar to that carried out by Mboto *et al.*, (2009).

OCB had the second largest zone of inhibition for 75% of the test bacteria with mean zone of inhibition ranging from 4.00-9.63mm, while AAP had the least zone of inhibition to all the test bacteria.
There were significant differences in the antibacterial activity of DAH herbal remedy to *Staphylococcus aureus*, than do the other herbal medicines going through the various rows in table 7. There were no significant differences in the antibacterial effect of OCB, UMU, NH2 herbal remedy, GBM and AMB to *Staphylococcus aureus* hence the superscript **b**. Along the *Streptococcus faecalis* row, there were no significant differences in the effect of CNB, OCB, EPL, UMU, UAE, NH2 herbal remedy, AMB and AAP to it.

NH2 herbal remedy was effective against *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeroginusa, Salmonella typhii and E. coli*, giving wide zones of inhibition going through the column of table 7. This was because natural honey was part of the ingredient used in preparing the herbal medicine. This is similar to a research carried out by Mboto *et al.*, (2009).

OCB and AMB were effective against *Staphylococcus aureus, Klebsiella pneumonia, Psedomonas aeroginusa, Proteus vulgaris, Salmonella typhii and E. coli* because aloe vera was part of the herbal plant used in preparing the herbal medicine. This is also similar to the work done by Tapsell *et al.*, (2006) and Mustapha *et al.*, (2009). Aloe vera has been known as herbal medicine for healing wounds, cuts, burns etc.

The concentrations of the various herbal medicines had a varying effect on the test bacteria. Table 9 showed that at 500mgml^{-1} the herbal medicine exhibited greater effect on the test bacteria. Meaning that according to one's body weight, one can double or reduce the concentration of the drug for effective treatment and to avoid intoxication.
The results presented in this study on the sterility of the herbal medicines showed that even though herbal medicines are specifically formulated and hygienically prepared to meet NAFDAC specification, there are incessant presences of environmental contaminants in the final product. This poses a great public health problem to the end users and called for routine supervision and/or inspection of these herbal drug manufacturers by SON-Standard Organisation

of Nigeria or NAFDAC for continual conformation to their standard at all times in order to cope or arrest these cases of contaminants.

In conclusion, I would strongly recommend the use of herbal medicine for use in the Nigeria health sector, since it has proved to be better in handling the multidrug resistant microbes, accessible and cheap. The phytoconstituents of the selected locally produced herbal medicines was determined and it showed that it have antimicrobial, antimutagenic, anticarcinogenic, antiproliferative and vasodilatory actions. The bacterial load of the selected locally produced herbal medicine were equally determined, in which that of UMU and UAE exhibited a bacterial load above that set by the World Health Organization, British Pharmacopoeia and the United States Pharmacopoeia, while that of the other herbal medicines were able to meet the recommended tolerable microbial limits in non-sterile pharmaceutical products. However, further phytochemical benefits of these herbal medicines should be look into, and further research should be carried out on the mycotoxins, pesticide and toxic heavy metal contaminants of the herbal medicines sold in Calabar, Nigeria.

REFERENCES

[1] Abba, D., Inabo, H.I., Yakubu, S.E. and Olonitola, S.O. (**2009**). *African Journal of Traditional, Complementary and Alternative Medicines (AJTCAM), 6(**1**): 70-77.*
[2] Adeshina, G. O., Noma S.T., Onaolapo, J.A., Ehinmidu, J. O. (**2010**). *International Journal of Pharmaceutical Research and Development, 2(**8**): 1-8*.
[3] Akinyemi, K. O., Oladapo, O.I., Okwara, C .E., Ibe , C. C., Fasure, K. A. (**2005**). *BMC complementary Alternative Medicine 35: 5-6.*
[4] Awafisayo, S. O., Udoh, I. E. and Mbagwu, H. O. C. (**2010**). *IJPI's Journal of Biothechnology and Biotherapeuatic, 1(**2**): 18-23.*
[5] Bukar, A., Uba, A. and Oyeyi, T. I. (**2010**). *Bayero Journal of Pure and Applied Sciences, 3(**1**):43-48.*
[6] Chandrappa, C.P., Schiplasheree, C.B., Karthik, M.R., Govindappa, M., Sadanmanda, T. S. (**2011**). *Journal of Phytology, 3(**1**): 26-32.*
[7] Cheesbrough, M. (**2002**). *Medical Laboratory Manual for Tropical Countries, ELBS ed*. Tropical Health Technology Publications, UK, Pp. 22-392.
[8] Duru, C.M. and Mbata, T.I. (**2010**). *Journal of Developmental Biology and Tissue Engineering, 2(**1**):1-4.*
[9] Egawaikhide, P.A. and Gimba, C.E. (**2007**). *Middle-East Journal of Scientific Research, 2(**4**): 135-138.*
[10] Farnsworth, N. R. (**1990**). The Role of Ethnopharmacology in Drug Development. In Chadwick, M. (ed). *Bioactive compounds from Plants.* John Wiley and Sons, New York. Pp. 321-69.
[11] Gafar, M. K., Hassan, L.G., Dangoggo, S.M., Hod, A. U. (**2010**). *Journal of chemical and pharmaceutical research, 2(**5**):277-285.*
[12] Mboto, C.I., Eja, M.E., Adegoke, A .A., Iwatt, G .D., Asikong, B. E., Takon, I., Udo, S.M. and Akeh, M.(**2009**). *African Journal of Microbiology research, 3(**9**):557-559.*
[13] Mboya, T.O. (**2003**). *Medical Research and Traditional Medicine.* Nov. 24-28, Nairobi, Kenya.
[14] Mustapha, K.A.H., Abdullahi, B.A., Rogo, L.D., Gaiya, Z.A. and Kumurya, A.S. (**2009**). *Bayero Journal of pure and applied sciences, 2(**1**): 34-40.*
[15] Mwambazi, W.C. (**1996**). WHO partnership in the Development and Utilization of Herbal Remedies in Ethiopia. In: Abebe, D. (ed). *Proceedings of the Workshops on Development and Utilization of Herbal Remedies in Ethiopia*. Addis Ababa. EHNRI 26-7.
[16] National Agency for Food, Drug Administration and Control (**2008**). *The Role of NAFDAC in Regulation of Herbal Medicines in Nigeria.* Central Drugs and Vaccines Control Laboratory (CDVCL), pp. 18-23.
[17] Ncube, N. S., Afolayan, A. J. and Okoh, A. I. (**2008**). *African Journal of Biotechnology 7**(12):*** 1797- 1806.
[18] Njoroge, G.N. and Bussman, R. W. (**2006**). *Journal of Ethnobiology and Ethnomedicine, 2(**54**):1-13.*
[19] Perumalsamy, R. and Ignaciumuthu, S. (**2000**). *Journal of Ethnopharmacology, 69:63-71*.
[20] Prescott, L.M., Harley, J. P. and Klien, D.A. (**2002**). Antimicrobial Chemotherapy In: *Microbiology, 5th Ed*. McGraw-Hill Higher Education Publishers, New York, Pp.806-822.
[21] Sofowora, E. A. (**1994**). *Medicinal Plants and Traditional Medicine in Africa*. University of Ife Press, Nigeria. Pp. 1-23.
[22] Tapsell, L.C., Hemphill, I. and Cobaic L. (**2006**). *Med. J. Aust. 185 (**4**):S4-24.*
[23] World Health Organization (**1993**). *Author Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines*, World Health Organization Regional Office for the Western Pacific, Manila. Pp.12-17.
[24] World Health Organization (**1998**). *Author Regulatory Situation of Herbal Medicines: A Worldwide review*. World Health Organization, Geneva. Pp.43-52.