ANTIMICROBIAL, PHYTOCHEMICAL AND HAEMOLYTIC EVALUATION OF KAMUE LOCAL CONCOCTION *IN VITRO*

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PREAMBLE

- Plants are the richest sources of drugs in traditional and mordern systems of medicine, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs(6). All over the world thausands of these plants have been selected for several therapeutic purposes as antibacterial and antifungal agents (10),
- The usage of plants in curing illnesses has deep roots in human history, in post colonization years, African communities showed great dependence on plants for medicine.
- Over the years, there have been alarming reports of multiple drug resistance by the medically important strains of bacteria and fungi(13, 1). The persistent increase in antibiotic resistant strains of organisms led to the development of more potent antibiotics as the 3rd and 4th generations of cephalosporins by pharmaceutical companies (11).
- Some of these plants are rich in a wide variety of secoundary metabolites as tannins,terpenoids, alkaloids flavonoids et.c which are found to posses antimicrobial properties (7). The major part of traditional therapy involves the use of plant extracts and their active constituents (2).
- Attention paid by health authorities to the use of herbal medicines has increased considerably because they are often the only medicines available to the rural populace of the less developed countries (4).

STATEMENT OF THE PROBLEM



Contaminated Water 1



Poor hygiene – Lack of Proper hand wash



Poor food handling



Contaminated Water 2



Typhoid Fever



Poor food handling



Fake or Substandard Drugs



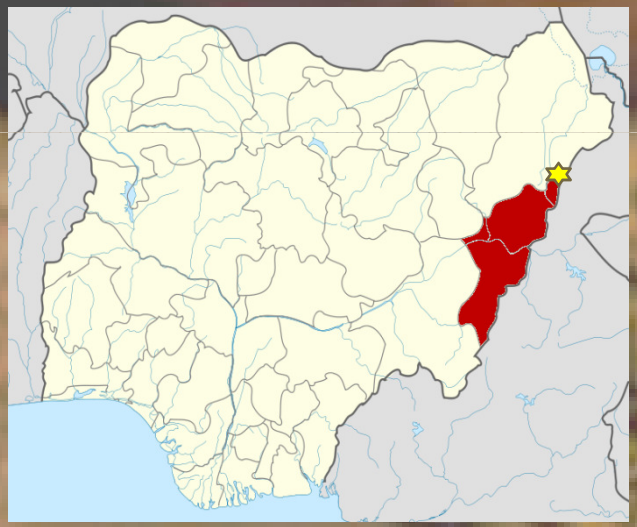
Poor sanitation



Inproper waste disposal

SCOPE OF THE STUDY

- The poverty levels in some of our communities and the lack of adequate health care facilities results to the use of traditional medicines for medicare.
- We choose a popular concoction used by the Kamue people of Michika in Adamwa State, North East Nigeria for the study.
- The choosen concoction is made up of the barks of the following medicinal plants remedy for the treatment of Typhoid fever.



THE MAP OF NIGERIA

ADAMAWA STATE, N. E.

MICHIKA -10^0 37` 5`` N, 13$^0$ 23`22`` E



1. Azadirachta indica
L/N - Kaswazoli



2. Anogeissus leiocarpus
L/N - Dheka



3. Khaya senegalensis
L/N - Chei



4. Vitellaria paradoxa
L/N - Fumua



5. Parkia biglobosa
L/N - Lughuni



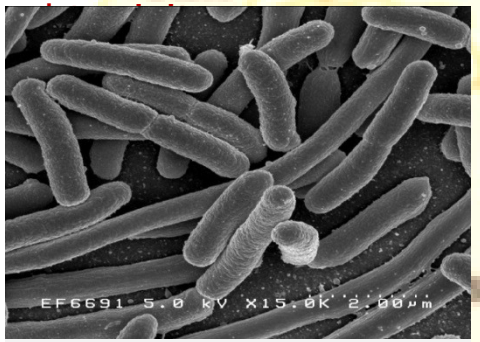
6. Red Potash
L/N – Bremi duzhi

AIMS AND OBJECTIVES OF THE STUDY

To investigate the efficacy of the concoction *in vitro,* on models of some infective microbes.

To attempt to identify the most active of the plant extracts.

To ascertain the probable side effects of the herbs on consummers based on dosage a



Escherichia. Coli



Pseudomonas species



Salmonella typhi



Flow chat showing the extraction of the medicinal plants.



Shade dried leaves



Grinded into powder

30 g powder + 700 ml distilled water.



Mixed and warm at 45-55 oC



Cooled and filtered/sieved with muslin cloth



Centrifuged for 10 mins

Separated with filter paper





Evaporated at 90 oC

Solid extract collected and weighed

Stored in sample bottle and refrigerated



ANALYTICAL METHODS

- **Phytochemical analysis**
  - ✓ Tests for tanins, steroids, phlobatanins, flavonoids, saponins, terpenoids, cardiac glycoside, anthraquinones and alkaloids were done using standard methods as described by Edeoga et.al (2005).

- **Elemental analysis of red potash**
  - ✓ Concentrations of Pb, Zn, Cu, Fe, Ca and Mg were estimated using atomic absorption spectrometer (AAS) in a 1:99 dilution. (Indrayan, 2000)

- **Antimicrobial activity**
  - ✓ Disc preparation of the standard antimicrobial agents (tetracycline, amoxile, chloramphenicol and ampicillin) and the plant extracts/remedy were made.
  - ✓ The aqueous extracts of medicinal plants were prepared in 16, 32, 64 and 128 mg/ml. This was done as described by Olurinola in (1996), and Hague et.al. (2011)

- **In Vitro Hemolysis test using human whole blood**
  - ✓ Human whole blood was diluted in sterile phosphate buffer saline (PBS) and 1 ml of medicnal plant extract and observed under a binocular microscope. (Malagoli, 2007)

RESULTS

TABLE 1: Result of phytochemical analysis on aqueous extracts of the kamue concoction

Phytochemical	*A.leiocarpus*	*V. paradoxa*	*P.biglobosa*	*K.senegalensis*	*A. indica*
Tannins	+++	+	++	++	+
Phloba tannins	-	++	++	+	+
Saponins	+++	+	+	++	+++
Flavonoids	-	-	-	-	-
Steroids	+	+++	+	++	++
Terpenoids	++	+++	++	++	+++
Cardiac Glycoside	-	+++	+++	++	+
Alkaloids	+	+	+	+	+
Anthraquinones	+++	++	++	+	+

Keys: +++ = Highest concentration, ++ = High concentration, + = Low concentration, - = absent

Table 2 Result of elemental analysis of red Potash solution

Element	ppm (conc)
Mg	0.231
K	0.834
Cl	0.021
Ca	ND
Zn	0.014
Fe	0.008
Cu	0.016
Pb	ND

Note: ND = Not detected

Table 3 Result of antibacterial effect of the control and the aqueous extracts of medicinal plants and red potash (16mg/ml) *in vitro*.

DC/SAMPLE	E. Coli	P. specie	S. typhi
CHL	+++	++	+++
TTC	++	+	+++
AMX	+	+	+
AMP	+	+	+
K. senagalensis	+	-	-
A. indica	+	-	-
P. biglobosa	-	-	-
V. paradoxa	-	-	-
A. leicarpus	++	+	+
Red potash	+	+	-
Whole concoction	+	+	+

Key: DC = Drug control: CHL = Chloramphenicol: TTC = Tetracycline: AMX = Amoxil: AMP = Ampicillin; + = about 1mm zone of inhibition, ++ = about 2mm zone of inhibition, +++ = about 3mm zone of inhibition, - = No inhibition

Table 4: Result of antibacterial effect of drug control and the aqueous extracts of medicinal plants and red potash (32mg/ml) *in vitro*

DC/SAMPLE	*E. Coli*	*P. specie*	*S. typhi*
CHL	+++	++	+++
TTC	++	+	+++
AMX	+	+	+
AMP	+	+	+
K. senagalensis	+	-	-
A. indica	+	+	+
P. biglobosa	-	-	-
V. paradoxa	+	-	-
A.leiocarpus	++	++	++
Red potash	+	+	-
Whole concoction	+	+	+

Key: DC = Drug control: CHL = Chloramphenicol: TTC = Tetracycline: AMX = Amoxil, AMP = Ampicillin, + = about 1mm zone of inhibition, ++ = about 2mm zone of inhibition, +++ = about 3mm zone of inhibition, - = No inhibition

Table 5: Result of antibacterial effect of drug control and the aqueous extracts of medicinal plants and red potash (64mg/ml) *in vitro*

DC/SAMPLE	*E. Coli*	*P. specie*	*S. typhi*
CHL	+++	++	+++
TTC	++	+	+++
AMX	+	+	+
AMP	+	+	+
K.senagaleusis	+	+	+
A. indica	+	+	+
P. biglobosa	-	-	-
V. paradoxa	+	-	+
A. leiocarpus	++	++	++
Red potash	+	+	+
Whole concoction	+	+	+

Key: DC = Drug control: CHL = Chloramphenicol: TTC = Tetracycline: AMX = Amoxil, AMP = Ampicillin, + = about 1mm zone of inhibition, ++ = about 2mm zone of inhibition, +++ = about 3mm zone of inhibition, - = No inhibition

Table 6: Result of antibacterial effect of drug control and the aqueous extracts of medicinal plants and red potash (128mg/ml) *in vitro*

DC/SAMPLE	E. Coli	P. specie	S. typhi
CHL	+++	++	+++
TTC	++	+	+++
AMX	+	+	+
AMP	+	+	+
K. senegalensis	+	+	+
A.indica	++	++	+
P. biglobosa	-	-	-
V.paradoxa	+	-	+
A. leiocarpus	+++	+++	+++
Red potash	+	+	+
Whole concoction	+	+	+

Key: DC = Drug control: CHL = Chloramphenicol: TTC = Tetracycline: AMX = Amoxil, AMP = Ampicillin, + = about 1mm zone of inhibition, ++ = about 2mm zone of inhibition, +++ = about 3mm zone of inhibition, - = No inhibition

Table 7: Haemolytic effect at various concentrations of the aqueous extracts of medicinal plants and red potash compound with distilled water

Sample	125mg (Conc)	63mg (Conc)	32mg (Conc)	16mg (Conc)
K. senegalensis	AA	AA	-	-
A. indica	SH	SH	-	-
P. biglobosa	A	A	-	-
V. paradoxa	AAA	AAA	-	-
A. leiocarpus	AAA	AA	-	-
Red potash	SH	SH	SH	SH
Whole concoction	SH/A	SH/A	-	-
Control	TH ?			

Key: A = Agglutination of red blood cells, SH = Shrinkage of red blood cells, TH = Total Hemolysis of red blood cells, - = No agglutination, shrinkage or hemolysis of red blood cells

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CONCLUSION

- **The Kamue concoction and individual medicinal plants, and red potash exhibit some degree of antimicrobial activity on *E. coli*, *P. species* and *S. typhi*,**
- ***A. leiocarpus*, *A. indica*, *K. senegalensis* and red potash may effectively treat *E. coli*, *P. species* and *S. typhi* related ailments that plague the Kamue community or any other,**
- **The side effects observed *in vitro* such as agglutination, cell shrinkage and possible lysis may be reduced or prevented all together as the result of the action of intestinal enzymes on the orally administered aqueous solution when it is ingested,**
- It is expected that the use of natural products as antimicrobial agent may probably not elicit the same hemolytic action as the conventional antibiotic.

RECOMMENDATION

- **Toxicological studies be performed.**









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