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PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITIES OF RED POTASH AND SOME MEDICINAL PLANTS USED IN KAMUE LOCAL CONCOCTION

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ABSTRACT

The aqueous extracts of the stem barks of *Azadirachta indica, Parkia biglobosa, Anogeissus leiocarpus, Vitellaria paradoxa and Kaya senegalensis*, red potash and their mixture, a Kamue anti-typhoid traditional medicine was investigated for phytochemical and antimicrobial activities on *Salmonella typhi, Eschericia coli and Pseudomonasspecies* using the disk diffusion assay. The results of the phytochemical investigation revealed the presence of anthraquinones, alkaloids, terpenoids, tannins, saponins, and steroids in all the plants examined. The presence of these substances is an indicator of the possible pharmacological property of these plants. The antimicrobial tests indicated that the individual extracts as well as the concoction containing the red potash that is used in Kamue community showed reasonable activity as antibacterial agent, the concoction had more broad effects on the microbes, indicating a synergy among the individual constituents of the concoction. Findings from this study therefore justified the use of this concoction in the traditional herbal medicine and may be a potential source of novel broad spectrum drug for treating bacterial diseases.

KEYWORDS: Kamue, Concoction, Red Potash, Phytochemical, Antimicrobial activity.

INTRODUCTION

Antimicrobials are used worldwide in human medicine, food, agriculture, veterinary and household products in some cases preservative and others as drugs. Plants have been a tremendous source of antimicrobials and for the discovery of new products of medicinal value for drug development (Bbosa and Mwebaza, 2014). Almost every community in the world has list of herbal remedies for the treatment of several diseases and different other pathological disorder (Katiyar *et al*., 2012). Clinical research has confirmed the efficacy of several medicinal plants for the treatment of gastric disorders, and basic scientific research has uncovered many of the mechanisms to explain their therapeutic effects (Chatterjee and Bandyopadhyay,

2014). A number of different chemicals derived from plants are important drugs in clinical use today (Srivastava *et al.,* 2009).

Among the 70,000 species of medicinal plants recognized all over the world, more than 500 types of economically important medicinal plants are found in Kamue community. Unfortunately, only few of them are used for their medicinal value. Thus, the antimicrobial activity of only a few are been exploited for the treatment of bacterial infections Namita, P. and Mukesh, R. (2012).

In the present study a concoction and its individual constituents used traditionally in the treatment of typhoid in Kamue communities of Michika Local Government Area of Adamawa State, Nigeria was investigated.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals, solvents and media used for this work were of analytical grade and were procured from reputed dealers.

Plant Materials

The stembark of the five medicinal plants namely: *Azadirachta indica* (kamue-kaswazoli); *Parkia biglobosa* (kamue - Lughuni); *Anogeissus leiocarpus* (kamue-Dheka); *Khaya senegalensis* (kamue-Chei); *Vitellaria paradoxa* (kamue-Fuma) and Red potash (kamue-Bramey) were obtained from Michika Local Government Area of Adamawa State, Nigeria in the month of May, 2012. They were identified and confirmed by Prof. Mohammad Saquib of the Biological Science Department, Adamawa State University, Mubi. Voucher samples were kept in the Faculty herbarium.

Preparation of Plant Materials

Fresh plant materials were collected and air dried under room temperature. The plants were pulverized and 30 g each of the powdered samples were thoroughly mixed with 700 ml distilled water**.** Themixtures were warmed for 1hr at 45-55ºC using an electric hotplate. They were allowed to cool to room temperature and separated using muslin cloth. The pH of each extract was determined using pH meter (JENWAY, Model 3505) and the extracts were centrifuged at10,000 rpm for 5 minutes. The supernatant determined was further separated using Whatman No. 1 filter paper. The pH reading of the filtrate was also obtained and the filtrates were evaporated to dryness on water bath (HH, W21.Cr4211) at constant temperature of 90ºC. Solid extracts were collected, weighed and stored in a sampling bottle for further analysis.

Phytochemical Analysis

Phytochemical analysis was carried out using standard procedures described by Edeoga *et al.,* (2005) and Khan *et al.,* (2011).

Elemental Analysis of Red Potash

The elemental analysis was carried out according to the method described by Indrayan *et al*., (2000) using atomic absorption spectrophotometer (Buck 210, Buck Scientific). 1% of the red potash solution was prepared in deionized water for the determination of Pb, Zn, Cu, Fe, Ca, K and Mg.

Antimicrobial Activity

Disc Preparation of the Standard Antibacterial Agents and Plant Extracts

Disc of standard antibacterial agents and plant extracts were prepared by the method described by Olurinola (1996). Disc of 6mm diameter were perforated from Whatman No.1 filter paper and sterilized for 30 min in the hot air oven at 60 ºC, the drugs (tetracycline, amoxile, chloramphenicol and ampicillin) were prepared at concentration of 30µg/disc each soaked into the discs and allowed to air dry in the oven and kept ready for use. The aqueous extracts were prepared at concentration of 16, 32, 64 and 128 mg/ml each and kept for further use.

Antibacterial Test

The modified agar disc diffusion method was used as described by Hague *et al.*, (2011).

Hemolysis Test

The method of hemolysis test as described by Malagoli (2007) was used.

RESULTS

The percentage yield of the extracts is shown on Table 1. The result of phytochemical screening of showed the presence of anthraquinones, alkaloids, terpenoids, tannins, saponins, and steroids (Table2). Screening for antimicrobial activity of the traditionally used medicinal plants revealed that the plant extracts and red potash had varying effects on the growth of the isolates and the plant extracts had different inhibiting strength on the test organisms (Tables 4, 5, 6, 7).

Table 1: Percentage yield of the aqueous extract of the bark of five medical plants and red potash

Sample	Family	Solvent	Part used	Weight of sample (g)	Sample yield (g)	Percentage yield (%)
Whole concoction		Aqueous		30	7.9	26.33
Anogeissus leiocarpus	*Combretaceae*	Aqueous	Bark	10	1.2	12.00
Vitellaria paradoxa	*sapotaceae*	Aqueous	Bark	10	1.6	16.00
Parkia biglobosa	*Fabaceae*	Aqueous	Bark	10	1.3	13.00
Khaya senegaleusis	*Maliaceae*	Aqueous	Bark	10	1.6	16.00
Azadirachta indica	*Maliaceae*	Aqueous	Bark	10	1.0	10.00
Red potash		Aqueous		10	9.0	90.00

Table 2: Phytochemical analysis of aqueous extract of five medicinal plants

Phytochemical	*Anogeissus leiocarpus*	*Vitellaria paradoxa*	*Parkia biglobosa*	*Khaya senegaleusis*	*Azadirachta indica*
Tannins	+++	+	++	++	+
Phloba tannins	-	++	++	+	+
Saponins	+++	+	+	++	+++
Flavonoids	-	-	-	-	-
Steroids	+	+++	+	++	++
Terpenoids	++	+++	++	++	+++
Cardiac Glycoside	-	+++	+++	++	+
Alkaloids	+	+	+	+	+
Anthraquinoues	+++	++	++	+	+

Keys: +++ = Highest concentration**;** ++ = High concentration**;** + = Low concentration**;** - = Absent

Table 3: Elemental analysis of red potash solution

Element	Red potash ppm
Mg	0.231
Ca	ND
Zn	0.014
Fe	0.008
Cu	0.016
Pb	ND
K	0.897

Note: ND= Not detected

Table 4: Antibacterial effects of the drug controls, aqueous extracts of five medicinal plants, red potash and mixture (**16mg/ml**) *in vitro*

DC/SAMPLE	*Escherichia coli*	*Pseudomonas specie*	*Salmonella typhi*
CHL	+++	++	+++
TTC	++	+	+++
AMX	+	+	+
AMP	+	+	+
Khaya senagaleusis	+	-	-
Azadirachta indica	+	-	-
Parkia biglobosa	-	-	+
Vitellaria paradoxa	-	-	-
Anogeissus leicarpus	++	+	+
Red potash	+	+	-
Whole concoction	+	+	+

Key: DC = Drug control;CHL = Chloramphenicol;TTC = Tetracycline;AMX = Amoxile; AMP = Ampicillin**;** + = about 1mm zone of inhibition**;** ++ = about 2mm zone of inhibition**;** +++ = about 3mm zone of inhibition**;** - = No inhibition; Whole concoction = blend of five plant extracts and red potash

Table 5: Antibacterial effects of drug controls, aqueous extracts of five medicinal plants aqueous extracts of medicinal plants, red potash and the mixture (**32mg/ml**) *in vitro.*

DC/SAMPLE	*Escherichia coli*	*Pseudomonas specie*	*Salmonella typhi*
CHL	+++	++	+++
TTC	++	+	+++
AMX	+	+	+
AMP	+	+	+
Khaya senagalensis	+	-	-
Azadirachta indica	-	+	+
Parkia biglobosa	-	-	-
Vitellaria paradoxa	+	-	-
Anogeissus leicarpus	++	++	++
Red potash	+	+	-
Whole concoction	+	+	+

Key: DC = Drug control; CHL = Chloramphenicol; TTC = Tetracycline; AMX = Amoxile; AMP = Ampicillin; + = about 1mm zone of inhibition; ++ = about 2mm zone of inhibition; +++ = about 3mm zone of inhibition; - = No inhibition; Whole concoction = blend of five plant extracts and red potash

Table 6: Antibacterial effects of the drug controls, aqueous extracts of five medicinal plants, red potash and mixture (**64mg/ml**) *in vitro*

DC/SAMPLE	*Escherichia Coli*	*Pseudomonas specie*	*Salmonella typhi*
CHL	+++	++	+++
TTC	++	+	+++
AMX	+	+	+
AMP	+	+	+
Khaya senagaleusis	+	+	+
Azadirachta indica	+	+	+
Parkia biglobosa	-	-	-
Vitellaria paradoxa	+	-	+
Anogeissus leicarpus	++	++	++
Red potash	+	-	-
Whole concoction	+	+	+

Key: DC = Drug control; CHL = Chloramphenicol; TTC = Tetracycline; AMX = Amoxile; AMP = Ampicillin; + = about 1mm zone of inhibition; ++ = about 2mm zone of inhibition; +++ = about 3mm zone of inhibition; - = No inhibition; Whole concoction= blend of five plant extracts and red potash

Table 7: Antibacterial effects of the drug controls, aqueous extracts of five medicinal plants, red potash and mixture (**128mg/ml**) *in vitro*

DC/SAMPLE	*Escherichia Coli*	*Pseudomonas specie*	*Salmonella typhi*
CHL	+++	++	+++
TTC	++	+	+++
AMX	+	+	+
AMP	+	+	+
Khaya senagalensis	+	+	+
Azadirachta indica	++	++	+
Parkia biglobosa	-	-	-
Vitellaria paradoxa	+	-	-
Anogeissus leicarpus	+++	+++	+++
Red potash	+	+	-
Whole concoction	+	+	+

Key: DC = Drug control; CHL = Chloramphenicol; TTC = Tetracycline; AMX = Amoxile; AMP = Ampicillin**;** + = about 1mm zone of inhibition**;** ++ = about 2mm zone of inhibition**;** +++ = about 3mm zone of inhibition**;** - = No inhibition; Whole concoction = blend of five plant extracts and red potash

Table 8: Hemolytic effect at various concentrations of the aqueous extracts of five medicinal plants and red potash compound and whole mixture with distilled water

Sample	125mg/ml	63mg/ml	32mg/ml	16mg/ml
Khaya senagalensis	AA	AA	-	-
Azadirachta indica	SH	SH	-	-
Parkia biglobosa	A	A	-	-
Vitellaria paradoxa	AAA	AAA	-	-
Anogeissus leicarpus	AAA	AA	-	-
Red potash	SH	SH	SH	SH
Whole concoction	SH/A	SH/A	-	-
Water (Control)		TH		

Key: A = Agglutination of red blood cells**;** SH = Shrinkage of red blood cells**;** TH = Total AA; AAA; Hemolysis of red blood cells; - = No agglutination, shrinkage or haemolysis of red blood cells.

DISCUSSION

In spite of being among well-known medicinal concoction used in Kamue traditional medicine to treat several ailments, studies' pertaining to the pharmacological properties of this concoction is scarce. Many medicinal plants in traditional use are considered to be potential antimicrobial drugs as well as source for unique compounds with anti-microbial activity, with possibly new modes of action (Doughari *et al*., 2009).

The antimicrobial potency of *A. leiocarpus* have been further corroborated by this study with its observed inhibitory activities on *E. coli*, *Pseudomonas species* and *S. typhi* in a concentration dependent manner as its inhibitory effects on the microbes increased as its concentration was increased from 16 mg/ml - 128 mg/ml (Tables 4, 5, 6, 7). The results obtained from this study agrees with the traditional practice of the use of *A. leiocarpus* as a medicinal plant and also is indicative that the plant may be efficacious as indicated by other traditional medical practitioners for the treatment of *helminithiasis*, *trypanosomiasis*, malaria and dysenteric syndrome. Other related species in the Anogeissus family such as *A. accuminata* have equally shown anti-inflammatory and analgesic properties (Hemamalini *et al*., 2010). *A. leiocarpus* have been reported to have significant antibacterial activities in both humans and animals (Mann *et al*., 2008; Mann *et al*., 2009; Kabore *et al*., 2010). Previously, a terpenoidal fraction of *A. leiocarpus* was reported by Mann *et al*. (2009) to possess significant antimicrobial activities *in vitro* against some of the bacteria implicated in the pathogenesis of human infections, it was also reported to show reverse transcriptase inhibitory activity in HIV- I (Rimando *et al*., 1994).

A. indica and *K. senegalensis* which formed part of the Kamue antibacterial concoction showed good antibacterial activity against all the micro organisms except at 16 mg/ml.

They have also been indicated by literature to possess significant biological activities namely anti-bacterial (Kubmarawa *et al*., 2008; Gajanan, 2012) antimalarial (Vandanal *et al*., 1991) anti-inflammatory and immunomodulatory (Biswas *et al.,* 2002) and antioxidant activities (Ghimeray *et al*., 2009).

The phytochemical screening of *Vitellaria paradoxa* revealed that the plant contained tannins, saponins, steroids terpenoids, cardiac glycoside, alkaloids and anthraquinoues, in agreement with the findings of Ndukwe *et al*., (2007) and El-Mahmood *et al.,* (2008). The antimicrobial activities of aqueous extract of this plant as performed against the isolates of *Staphylococcus aureus, Escherichia coli* and *Pseudomonas specie* exhibited inhibitory activity only on the growth of *E. coli* at concentrations above 16 mg/ml. This work is in agreement with the findings of El-Mahmood *et al*., (2008) that the aqueous extract of *Vitellaria paradoxa* had the least antimicrobial inhibitory activity compared to the acetone and ethanol extracts.

The traditional use of red potash commonly called “jan kanwa” in Hausa language is mainly for medicinal purposes in both human and in veterinary medicine, where it is used as an additive. Considering its nature as a salt and the result from this research showed that there is no doubt, it has some considerable antimicrobial properties in varying concentration.

The hemolytic properties of the mixture and five medicinal plants (*A. leiocarpus, V. paradoxa, A. indica, K. senegalensis,* and *P. bioglobosa*) red potash were evaluated *in vitro.* This property was compared with haemolytic property of distilled water. The study showed very strong agglutination in the 128 and 64 mg/ml *for K. senegalensis, V. paradoxa* and *A. leiocapus* (Table8). *P. biglobosa* shrank the red blood cells within 128 and 64 mg/ml concentration, the concoction also showed the same effect. The effects of these aqueous extracts on the blood cells cannot be linked authoritatively to the antimicrobial effects *in vitro* but it may not be unconnected to the effects observed. The same can be said for red potash solution being the only substance which showed some activity with all the doses used (16 – 128 mg/ml) (Table 8). The elemental composition of the five medicinal plant extracts and red potash may have acted individually or in synergy with one another on the cell membrane of the red blood cells, or the microbes as well. It is worthy of note however, that the traditional uses of salts which is mainly as sterilizers, astringents, preservatives justifies the use of red potash in the concoction as acid neutralizer considering the mixture of phytochemicals from the medicinal plant and also as an enhancer of antimicrobial drug.

Recent outcry of multi drug resistance in human and animals by pathogenic bacteria including adverse side effects of many antibiotics has raised the enthusiasm in the search for new antimicrobial compounds from medicinal plants, leading to several reports of same from around the world (Shibata, 2000).

Antimicrobials may have a significant clinical value in treatment of infections, particularly those from the aqueous extracts of plants, and these have formed the nucleus of many application for instance in foods, pharmaceuticals, alternative medicine and natural therapies. Studies have shown that higher plants have been shown to be potential source for new antimicrobial drugs.

Further research should have traditional medicinal plants as focus on the search for potential antimicrobial compounds.

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