International Journal of Advances in Engineering and Management (IJAEM)
Volume 2, Issue 7, pp: 629-635 www.ijaem.net ISSN: 2395-5252

Antibacterial, Antiplasmodial and Acute Toxicity Potentials of Methanolic Extract of Staudtia kamerunensis Stem Bark

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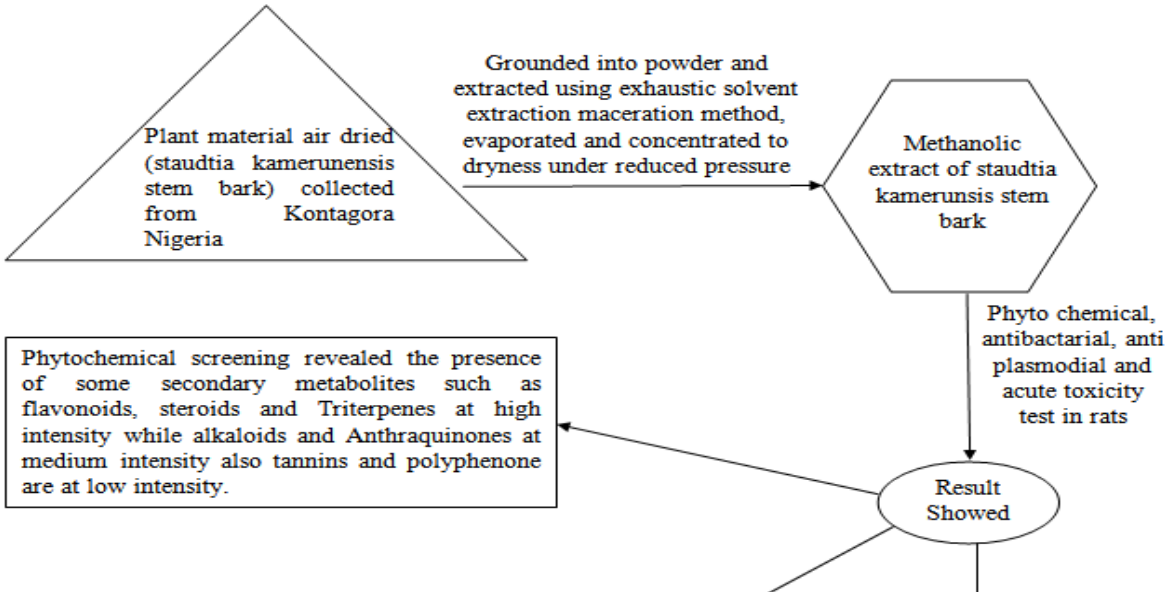
Date of Submission: 25-09-2020 Date of Acceptance: 12-10-2020

ABSTRACT: In this study, Antibacterial and anti-plasmodia activities of the methanolic extract of Staudtia kamerunensis stem bark against some pathogenic microorganisms and P. bergei was investigated**.** Phytochemical studies (GC-MS RT profiling) revealed the presence of some secondary metabolites. The extract was tested against seven bacterial strains both Gram-positive (Mycobacterium smegmatis, Staphylococcus aureus, Bacillus subtilis, Peptostreptococcus asaccharolyticus) and Gram-negative strains, (Klebsiella Pneumonia, Escherichia coli, Salmonella typhi). Minimum inhibitory concentration and the minimum bactericidal concentration of the extract showed activities against Mycobacterium smegmatis, Klebsiella Pneumonia, Salmonella typhi, Staphylococcus aureus and Peptostreptococcus asaccharolyticus. The extract demonstrated high safety with LD_{50} value higher than 5000mg/kg body weight. The extract shows a potent on anti-plasmodial activities with P. bargie inhibition of 41.73%. The results demonstrated that Staudtia kamerunensis stem bark extract can be used as a source of cheaper, less toxic novel antibiotic and anti-malarial substances for drug development.

Keywords: Medicinal plants, Antibacterial, Anti-plasmodial, Acute toxicity, GC-MS RT, Staudtia kamerunensis

GRAPHICAL ABSTRACT



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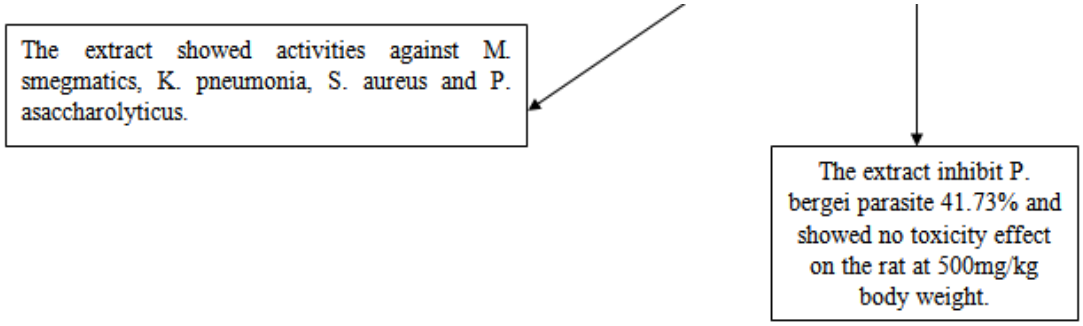

I. INTRODUCTION

The bioactive compounds contained by plants necessitate the need for phytochemical evaluation in medicinal plants for potentials application in the treatments of human ailments as alternatives [1]. Drug resistance parasite has rendered most of the drugs used in treating many human diseases ineffective [2], and this required urgent need and continuous search for new drugs from natural sources as most of the drugs used are either derived from plant or end-product of the natural source [2-4]. Staudtia kamerunensis from myristicaceae family is a large evergreen tree with a roundish crown. The plant can grow 35 meters tall and the bole which has narrow buttresses is up to 2 meters tall, and up to 75 cm in diameter [5]. The tree is harvested from the wild for local medicinal use and for timber, which is used locally and also traded. plant that is used medically by the West Africa people especially Eastern part of Nigeria, Ivory Coast and Cameroon, DR Congo and Central Africa where the plant is found in abundance [5].The bark decoction of the plants part is taking orally in treating many ailments such as treating dysentery, lung complaints and cough [5]. The stem bark decoction in DR Congo, they are given to children to drink or applied as enema against cough and applied as a rub to treat skin diseases, oedema and wounds [5].Medicinal plants are parts of some African diets and are very important to their health. Traditional medicine began when man started searching for food in the bush by ploughing and eating all types of leaves and fruits [6]. Natural product compounds are also of great interest in the process of drug discovery and design. Mainly plant-derived natural product constituents have long been sources of drugs. Large proportion (30–40%) of the pharmaceuticals available in modern medicine is directly or indirectly derived from natural sources [6, 7]. Plants are used as primary source of medicinal agents due to its availability and cheapness by 80% of the world's population [8]. Some of the diseases treated using drugs from plant include bacterial infection and malaria. These drugs include Artemisinin derived from the Qinghao plant (Artemisia annua L, China 4th century) and quinine from the cinchona tree (South American, 17th century), penicillin found in 1947, methicillin found in 1959, tetracycline found in 1948, erythromycin found in 1952 and streptomycin found in 1943 [9-10, 11-13].

This research was carried out because there is little information and report on this plant.

II. EXPERIMENTAL

Sample collection and Preparation

The stem bark of Staudtia kamerunensis was collected from the eastern Nigeria in March 2017. Identification was done by Mr. Idris M. Sabi, Department of Forest Resources Management, Forestry Research Institute of Nigeria and Mr. Mukaila Yusuf, Department of Forestry , Federal College of Wildlife Management, New Bussa, Niger State, Nigeria with voucher specimen no: [Staudtia kamerunensis (Musa / KNT / FHI: 1483)] was dropped at the institute.

Extraction and Isolation of the plant's extract

The stem bark (1kg) was air-dried at 37^{o}C and ground to powder. Extraction was carried out following the method described by [14] with slight modification.

Crude Extracts Acute Toxicity Tests. Acute toxicity of the plant's extract was tested using oral administration method. The oral administration of the plant's extract at a single high dose of 5,000 mg/kg body weight was carried out according to OECD methods [15].

Anti-Plasmodia Screening of the Extracts
Inoculation of Parasite

Highly parasitized (20-30% parasitemia) blood was obtained by cardiac puncture from Plasmodium berghei infected mice. The blood was diluted with phosphate buffer saline and 0.2ml of

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the diluted blood was intraperitoneally inoculated into the mouse [16].

Treating of Inoculated Mice with Plant Extracts

Four days (4) suppressive test were carried out to evaluate the antimalarial properties of the extracts according to the method described by [17]. The PCV was determined using the microhaematocrit method as described by [18].

Collection of Blood and Preparation of Serum

The collection of blood samples for biochemical analyses was done as described by [19].

Biochemical Parameters

Aspartate amino transferase activity (AST)

This was carried out based on the procedure [20].

Alanine aminotransferase activity (ALT)

This was carried out based on the procedure [20].

Alkaline phosphatase (ALP)

It was carried out as described by [21].

Determination of Serum Total Protein Concentration

Serum total protein concentration was calculated using Randox kit from Randox Laboratories Limited, U. K. [22].

Antibacterial Assay

The antibacterial assay of the crude extract was evaluated using disc diffusion [23-25]. Seven strains of bacteria consist of Gram-positive bacteria: B. subtilis (ATCC NO.19659TM), S. aureus (ATCC NO.25923TM), M. smegmatis (ATCC NO.14468TM), P. asaccharolyticus (ATCC NO.14963TM) and Gram negative: K. pneumonia (ATCC NO.12228TM), S. typhi (ATCC NO. 29212TM), andE. coli (ATCC NO.25922TM).

III. RESULTS AND DISCUSSION

The Phytochemicals screening of the methanolic extracts of Staudtia kamerunensis in Table 1 shows that there was a mild content of polyphenone and Tannins, medium contents of alkaloids, saponins and Anthraquinones. The extract gave a high intensity of flavonoids and Steroids &triterpenes. These secondary metabolites may be responsible for some of the activities of the extract.

Table1: Phytochemical screening of Staudtia kamerunensis methanolic extract

Plant extract	Alkaloid	Saponins	Phenolic; Anthraquinones	Flavonoids	Polyphenone	Tannins	Steroids & Triterpenes
SkM04	++	++	++	+++	+	+	+++

+ = mild, ++ = medium, +++ = high intensity

%yield of SkM04 extracts was 20**%.**

Total protein content

Table 2 related the infected, untreated mice have lower serum alkaline phosphatase, total proteins and albumin concentrations when compared with the normal control, standard and extract-treated groups. However, mice treated with SkM04 recorded higher protein concentrations compared with the negative control group. Alanine transaminase (ALT) activities, on the other hand, were much higher in the untreated control. Treatments with the compound significantly lower activities of this enzyme towards normalization. Total protein content of the mice after treatment with the extract of SkM04 is 28.89±1.76mg/dL. The biochemical parameters monitored in the liver and serums are useful 'markers' for assessing tissue damage [26]. AST and ALT are markers of liver damage and can be used to assess liver cytolysis during parasitic infection [27]. In the present study, infected untreated mice have lower serum Alkaline phosphatase, total proteins and albumin concentrations when compared with the normal control, standard and extract-treated groups.

Alkaline phosphatases are often used to assess the integrity of the plasma membrane and endoplasmic reticulum [28]. The alteration in serum ALP activities in P. bergei infected untreated rats suggested that the integrity and functionality of endoplasmic reticulum and plasma membrane have been comprised by the malaria infection [29]. The total proteins and albumin play major roles in assessing the integrity of kidney and liver [30]. The study shows significant decrease which may be due to the mobilization of defensive enzymes (which are known proteins) to counter the effect of parasite-induced oxidative stress which were consequently ameliorated by some of the plant's extracts. The decrease in albumins and total proteins reported in this study could lead to over hydration which is injurious to cellular homeostasis. This will harmfully compromise the normal metabolic activities of the liver and consequently the health of the animals [31].The improvement in the concentrations of albumin and total proteins in rats that are cured with the plant's extract is an indication of the reduced pathological effect of the parasite.

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Table 2: Total protein contents of the mice

Extracts	Albumin (mg/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Proteins (mg/dL)
SKM04	5.62±0.25	13.16±0.35	36.9±1.90	190.56±4.49	28.89±1.76
Negative	2.32±0.32	25.04±0.50	49.6±0.36	134.05±3.90	21.34±0.91
Normal control	5.20±0.3452	29.68±0.78	38.9±0.42	178.43±0.97	49.78±1.89
Standard control	5.17±0.8571	37.6±0.79	68.4±0.67	189.05±1.37	52.83±3.57

Data are Mean ± SEM of duplicate determination, SkM04= Methanolic extract of Staudtia kamerunensis

Infected mice body weight changes

The body weight change in P. berghei infected mice following treatment with extracts Staudtia kamerunensis, is shown in Table 3. There was a significant decrease in body weight of all the experimental animals after induction. Treatment of the infected mice with 5mg/kg bw chloroquine (standard drug) increases the bodyweight of the animals at the end of the experiment however, infected untreated mice show a significant decrease in body weight of the mice. SkM04 extracts decrease the weight of animals after treatment.

Table 3: Effect of plant extracts body weight changes in P. berghei infected mice

Extracts	Before Inoculation	After inoculation	After treatment
SkM04	19.50±0.50	18.00±0.00	18.50±1.50
Control	19.50±2.50	21.50±1.50	24.00±1.00
Negative	24.00±1.00	21.50±0.50	18.00±3.00
Standard control	23.50±1.50	20.00±1.00	23.00±1.00

Data are Mean ± SEM of duplicate determination

Packed Cell Volume

The packed cell volume (PCV) in P. berghei infected mice (Table 4) following treatment with 5000 mg/kg, Staudtia kamerunensis methanol extracts SkM04 which indicated acute toxicity of LD_{50}>5000 mg/kg. There was a significant decrease in packed cell volume (PCV) of all the experimental animals after induction. The infected mice was treated with 5mg/kg body weight (bw) chloroquine (standard drug) and the resulted animal showed an increase in the packed cell volume (PCV), however, infected untreated mice show a decrease in packed cell volume (PCV) of the animals. However, the plant's methanolic extract increases the PCV of treated mice from 29.50±1.04to 31.50±1.50%.

Table 4: Effect of plant extracts on packed cell volume (PCV) in P. berghei infected mice

	Packed Cell Volume (%)		
Extracts	Before Inoculation	After inoculation	After treatment
SKM04	43.32±7.45	29.50±1.04	31.50±1.50
Negative	41.26±1.90	21.50±3.23	21.78±1.45
standard control	45.56±0.73	30.50±2.50	39.50±2.45

Data are Mean ± SEM

Acute Toxicity

The Staudtia kamerunensis extract shows sign of erythraemia upon acute (5000mg/kg bw) but, there is no death of any animal as shown in Table 5.

Table 5: Acute toxicity profile of some plant extracts

	observation (5000) mg/kg bw	**Mortality**	**LD_{50} (mg/kg)**
SkM04	Erythraemia	Nil	>5,000

Parasitaemia

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Table 6 presented the parasitaemia counts of P.berghei infected mice treated with extracts from Staudtia kamerunensis, plants. Treatment of the infected mice with 5mg/kg bw chloroquine (standard drug) produce significant antiplasmodial activities with 97.39% inhibitions of the parasite. Similarly, treatment with medicinal plant produces varying degree of antiplasmodial effect with percentage parasite inhibition of 41.73%.

Table 6: Effect of plant extracts on parasitaemia count in P. berghei infected mice

Parasitaemia				
Plant's extract	One	Three	Five	% Parasite Inhibition
SKM04	4.50±1.50	24.00±2.00	33.50±1.50	41.73
standard control	5.00±3.00	11.50±0.50	1.50±0.03	97.39

Data are Mean ± SEM of triplicate determination. The mean parasite inhibition with different superscript alphabet are significantly ($p<0.05$) difference

Antibacterial activity of SkM04 extract.

When the ratio of MBC/MIC value is lower than 2, the extract exhibit a bactericidal effect [27]. The activity of the antibacterial can be considerate when the diameter of inhibition zone observed is 9mm or more around paper disk [32].

In Table 7, the extract showed high susceptibility of 19mm against B. subtilis, K. pneumonia and S. typhi. It also show inhibition zone of 15mm against P. asaccharolyticus and 13mm against M. smegmatis. The extract has no activity against E. coli and S. aureus. The plant extract under study is traditionally used for the treatment of various bacterial infections which includes gastrointestinal (B. subtilis, S. typhi), respiratory (K. pneumonia, B. subtilis), skin (M. smegmatis) female pelvic and reproductive organs (P. asaccharolyticus) and urinary (B. subtilis) pathogens contributes to its validity as traditional treatments for such ailments. The sensitivity of these bacterial representatives to the studied extract is concentration-dependent. The extract MIC values as shown in Table 7was able to inhibit some gastrointestinal pathogens gastrointestinal like (B. subtilis, S. typhi), respiratory (K. pneumonia, B. subtilis) skin (M. smegmatis), female pelvic and reproductive organs (P. asaccharolyticus) and urinary (B. subtilis). This result has validated the use of the decoction of this plant in treating cough, respiratory diseases, gastrointestinal diseases, female reproductive organs infections etc [5]. The antibacterial activity has shown that the extract has a broad spectrum [33] since it inhibits both gram-positive and gram-negative bacteria strain.

Table 7: Antibacterial activity of SkM04

ORGANISM	Susceptibility (mm)	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC ratio	STM	%Yield
Gram-positive						20%
B. subtilis	19	25	25	1	16	
S. aureus	NA	NA	NA	NA	256	
M. smegmatis	13	50	100	2	<4.00	
P. asaccharolyticus	15	50	100	2	128	
Gram-negative						
K. pneumonia	19	25	50	2	25	
S. typhi	19	25	50	2	25	
E. coli	NA	NA	NA	NA	64	

NA = No Activity

IV. CONCLUSIONS

The plant under study is traditionally used for the treatment of various bacterial infections which includes gastrointestinal (B. subtilis), respiratory (K. pneumonia, P. aeruginosa, B. subtilis), skin (M. smegmatis) and urinary (P. aeruginosa, B. subtilis) pathogens contributes to its validity as traditional treatments for such ailments.

The methanolic extract showed activity of 41.73% against plasmodium berghei with little toxicity and also exhibit excellent activity against some of the pathogens, may be due to the presence of certain terpenes, flavonoids, saponins and other secondary metabolites identified in the stem bark.[33 and 34]. Further studies involving isolation of active compounds, future phytochemical studies and toxicity assays will lead to the development of other effective drugs for the treatment of bacterial infections. The results demonstrated that Staudtia kamerunensis bark methanolic extract can be used as a source of cheaper and less toxicity novel antibiotic and antimalarial substances for drug development.

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