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ANTIMICROBIAL SCREENING AND THE TOXICITY OF THE AQUEOUS EXTRACT OF THE ROOT OF *Zanthozylum zanthozyloides* ON HAEMATOLOGY AND THE SPLEEN OF WISTAR RATS

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Abstract
This study examines the antimicrobial and toxicity of aqueous extract of the root of *Zanthozylum zanthozyloides* on haematological parameters such as red blood cell (RBC), packed cell volume (PCV), Platelets (PLT).
Fourty (40) male and female adult wistar rats weighing between 115 and 245g were used for acute (7 day administration) and chronic (28 days administration) toxicity studies. The animals were divided into eight groups; group 1, 2, 3, 5, 6, and 7 were administered 500mg/kg, 1000mg/kg, and 2500mg/kg respectively while Groups 5 and 8 were used as control and were administered distilled water. The toxic manifestation, mortality, body weight changes were monitored during the period of administration and the result showed significant ($P < 0.05$) increase in body weight with resultant increase in haematological parameters (RBC, PCV) and decrease in platelets (PLT). The antimicrobial screening of the extract of *Z. zanthozyloides* showed zones of inhibition against S. epidermis (12.33 mm), *P. vulgaris* (14.33 mm), *R. oryzae* (11.33 mm) and maximum inhibition against *E. coli* (10.00 mm) while insignifantly ($P > 0.05$) inhibited *Klepsiella spp.* (8.33 mm), *P. chrysogenum* (11.33 mm) and S. cerevisiae (11.00 mm).
The significant increase in body weight of the animal resulting to increase in haematological parameters indicates that there is no significant damage recorded on the spleen organ analysed, while the antimicrobial screening reviews that *Z. zanthozyloides* could be used as a broad spectrum antibiotics as it inhibits the growth of both bacteria and fungi organisms.
This study showed that the aqueous extracts of *Z. zanthozyloides* could be used as a broad spectrum antibiotics as it inhibits the growth of both bacteria and fungi organisms. The aqueous extract *Z. zanthozyloides* significantly ($P < 0.01$) increased the body weight of the test animals for 28 days administration, causing increase in the haematological parameter such as RBC, PCV and decrease PLT which were significant at ($P < 0.01$, $P < 0.001$) for 7 days administration.

Introduction

The use of, and search for, drugs and dietary supplements derived from plants have increased in recent years. Pharmacologists, microbiologists, botanists, and natural-products chemists are combing the Earth for phytochemicals and leads that could be developed for treatment of various diseases. [1]

Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80 percent show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived. [1] More than two thirds of the world's plant species at least 35,000 of which are

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estimated to have medicinal value comes from the developing countries. At least 7,000 medical compounds in the modern pharmacopoeia are derived from plants[1]. In many medicinal and aromatic plants (MAPs) significant variations of plants characteristics have been ascertained with varying soil traits, and the selective recovery and subsequent release in food of certain elements have been demonstrated. [2] Great attention must be paid to choose soil and cropping strategies, to obtain satisfactory yields of high quality and best-priced products, respecting their safety and nutritional value.

Zanthozylum genus belongs to the Rutaceae family. It is economically important because of their alimentary, industrial and medicinal applications [3-4]. The plant is known for its antioxidative, anti-inflammatory, antisickling, antibacterial, antiviral, antihepatoxicity, antiallegic, antitumoral and antihypertensive properties [5-7]. The methanol extract preparation of the powdered root of *Z. zanthoxyloides* containing flavonoids, chelery-thrine, berberine and phenol canthine-6-one have been reported to possess strong antibacterial activity [8-9]. They have been used as components of antiseptic, antiparasitic and analgesic preparations for managing small pox, syphilis and related disease conditions [10].

Species of this genus are of economic importance as sources of edible fruits, oils, wood, raw materials for industries, medicinal plants, ornamentals, culinary applications, and are characterized by a satin wood commonly used in woodworking [11,3]. In Africa the wood of *Z. gillettii, Z. tessmannii, Z. lemairei and Z. leprieurii* are used in construction of houses, drums and ships and for decorative woodwork, carpentry, and paper industry[11,3]. In some countries of this continent, root bark and stem of many species of *Zanthozylum* are used as a vermifuge, febrifuge and piscicides production [2].

This study was designed to evaluate the Anti-microbial screening and the toxicity of the aqueous extract of the root of *Z. zanthozyloides* on (haematological parameters) red blood cell, packed cell volume, platelets and the effect on the spleen on animal model.

Materials and Methods

Preparation of Extract:

Z. zanthozyloides root was washed carefully with distilled water, shade-dried for several days to preserve the volatile material of the plant, chopped into small pieces and further shade-drying was carried out for seven days before milled into powder using electric blender (Blender III, Model MS-223; Tapai Taiwan). The powder was kept in an air-tight polythene bag and was stored in cool dry place prior to the usage. The powdered sample of *Z. zanthonxyloides* root was extracted by weighing 650g of the powder and soaked in eight (8) Litres of distilled water in a plastic vial and was agitated at six (6)hours intervals for a period of seventy two hours, thereafter was sieved using a clean muslin cloth. The filtrate collected was concentrated with the aid of steam by heating over water bath at a constant temperature (100^0C). At the end of the concentration, the resultant yield extract was weighed and percentage extract was calculated, and then stored in an air-tight jar for further analysis.

Test Organisms

The test organisms includes *Proteus vulgaris, Staphylococcus epidermis, Escherichia coli, Staphylococcus aureus, Klebseilla pneumonaiae, Candida albicans, Penicillium chrysogenum, Rhizopus oryzae, Saccharomyces cerevisiae* and was obtained from Medical Microbiology Laboratory, of University of Benin Teaching Hospital (UBTH), Benin City, Nigeria.

Microbial Inoculum Preparation for Susceptibility Testing

The inoculants were prepared by inoculating the test organisms in nutrient broth and incubate for 24 hours at 37^0C. After incubation, one milliliter of the cultures were inoculated onto solidified nutrient agar using a Pasteur pipette. These procedures were repeated using potato dextrose broth for fungi and were incubated at 28^0C for 72 hours.

Antimicrobial assay

Antimicrobial activity was evaluated by noting the zones of inhibition against the test organisms following the method used by[24]. Two colonies of a 24-hour plate culture of each organism were transferred aseptically into 10ml sterile normal saline in a test tube and mixed thoroughly for uniform distribution. A sterile cotton swab was then used to spread the resulting suspension uniformly on the surface of oven-dried nutrient agar and potato dextrose agar plates for bacteria and fungi, respectively. Three (3) adequately spaced wells of diameter 4mm per plate were made on the culture agar surface respectively using sterile metal cup-borer. 0.2ml of each extract and control were introduced in each hole under aseptic condition with the aid of pipette pump, kept at room temperature for 1 hour to allow the agents to diffuse into the agar medium and incubated accordingly. Conventional antibiotics were used as positive controls for bacteria and fungi respectively; distilled water was used as the negative control. The plates were incubated at 37^oC for 24 hours for the bacteria strains and at 28^oC for 72 hours for fungal isolates. The zones of inhibition were measured and recorded after incubation. Zones of inhibition around the wells indicated antimicrobial activity of the extracts against the test organisms. The diameters of these zones were measured diagonally in millimeter with meter rule and the mean value for each organism from the triplicate cultured plates was recorded. Using the agar-well diffusion technique, an already made gram positive and gram negative (Asodisks Atlas Diagnostic, Enugu, Nigeria) standard antibiotic sensitivity disc purchased from a laboratory chemical equipment store in Benin City was used as positive control for bacterial while Metronidazole was used as positive control for fungi. Sterile distilled water was used as negative control for all the test organisms.

Sub-Acute Toxicity Study

Forty (40) male and female adult rats weighing between 115 and 245g were used for the sub- acute toxicity studies and were housed under standard laboratory conditions (12hr day/night cycle). The animals were divided into eight groups; I, II, III, IV, V, VI, VII, VIII respectively. Group I, II, III and were administered 500mg/kg, l000mg/kg, 2500mg/kg and group IV distilled water (as control) for twenty-eighty (28) days, while group V, VI, VII and were administered 500mg/kg, l000mg/kg, 2500mg/kg and VIII distilled water (as control) for seven (7) days respectively. The toxic manifestations, mortality; body weight changes, food and water intake were monitored during the period of

administration. The animals were fed with standard laboratory feeds (pellet) and water. Each rat in various groups were orally administered the required dose of the extract with oral gastric tube daily. At the end of monitoring period, the animals were sacrificed under anaesthetized condition. The blood was collected from the abdominal aorta for haematological and spleen for histological analysis. The haematological and histological parameter evaluated includes; red blood cell (RBC), packed cell volume (PCV), platelets (PLT) and the effect of the extract on spleen of the albino rats were established.

Results

The study of the antimicrobial screening of *Z. zanthozyloides* root extract revealed a significant ($P < 0.05$, $P < 0.01$, $P < 0.001$) inhibition against various organisms used as shown in table 1. The antimicrobial potency of the commercial antibiotics was not significantly different from the extract but for the activity against *K. pneumonaiae*. Where the aqueous root extract exhibited more inhibition as shown in Figure 2a.

The aqueous root of *Z. zanthozyloides* significantly ($P < 0.05$) increased the body weight of the animals and organ (spleen) body weight ratio for the 28 days administration while decreases in body weight was observed for 7 days administration. The result also shows significant ($P < 0.01$, $P < 0.001$) alteration in haematological parameters evaluated for 7 days; the body system showed an appreciable regulation of these alteration to an insignificant level compared with the control (Table 3a and 3b).

The examination of the histology of the spleen for twenty-eight (28) days administration revealed the effect of the extract on the spleen organ (figure 2a, 2b, 3a and 3b).

Discussion

The methanolic and ethanolic extract of *Z. zanthozyloides* have been reported by many authors to inhibit many microorganisms [2,5,6]. The result of this study showed the antimicrobial activities of aqueous extracts against bacteria and fungi organisms revealed significant ($P < 0.01$) zones of inhibition against *S. epidermidis* (12.33 mm), *P. vulgaris* (14.33 mm), *R. oryzae* (11.33 mm) and maximum inhibition against *E. coli* (10.00 mm) while insignificantly ($P > 0.05$) inhibited *Klebsiella spp* (8.33 mm), *P. chrysogenum* (11.33 mm) and *S. cerevisiae* (11.00 mm) at 100mg/ml concentration, although the concentration ranges from 100 to 3.125mg/ml as shown in Table 1. The result corroborates the publication on the antimicrobial activities of methanolic extract of *Z. zanthozyloides* against *S. aureus* (12.33 3.6 mm), *E. coli* (15.38 2.7 mm), *B. subtilis* (17.64 0.6 mm) and *P. aeruginosa* (14.42 1.9mm) [1,2,5,6]. The antimicrobial potential from this study was compared to some commercial antibiotics and antifungal agent which showed insignificant ($P > 0.05$) different against the various test organism (Figure 1a, 1b, 1cand 1d). The extract showed more effective inhibition against *Klebsiella* spp when compare to Augmentin (AU), Ceporax (CEP) and Amplicin (PN) as no zone of inhibition was recorded from the graph (Figure 1c), indicating that the *Klebsiella* spp. was resistant to the enlisted antibiotics but susceptible to the aqueous root extract of *Z. zanthozyloides*. This indicates that the extract have the capacity to inhibit both bacteria and fungi organisms as broad spectrum antibiotics as earlier reported.

Table 1: Zone of inhibition of the aqueous root extract of *Z. zanthoxyloides* on oral microorganisms

Organisms	Concentration (mg/ml)							
	100	**75**	**50**	**25**	**12.5**	**6.25**	**3.125**	**Distilled water**
Staphylococcus epidermidis	12.33^{a}±2.08	11.33^{a}±1.53	9.0^{b}±0.0	8.67^{b}±0.58	8.0^{b}±1.73	8.0^{b}±1.0	7.33±1.15	0.00
Eschericia coli	10.0^{a}±1.00	10.0^{a}±0.00	9.33^{a}±1.15	7.67^{b}±0.58	7.0^{b}±1.00	7.0^{b}±0.00	6.33^{b}±0.58	0.00
K. pneumonaiae	10.33±0.58	9.88±1.73	9.70±0.00	9.67±0.58	9.33±1.53	8.67±2.08	7.67±1.15	0.00
Staphylococcus aureus	9.0±0.00	8.43±0.00	8.35±0.58	7.67±2.31	7.21±1.53	6.33±1.53	5.33±1.15	0.00
Proteus vulgaris	14.33^{a}±2.08	13.67^{a}±3.21	13.33^{a}±0.58	10.33^{b}±3.21	10.0^{c}±0.00	8.67^{c}±0.58	8.00^{c}±1.00	0.00
Candida albicans	8.33±0.58	8.00±2.00	8.00±0.00	7.67±1.53	7.25±2.52	6.33±1.53	5.67±0.58	0.00
Rhizopus oryzae	11.33^{a}±2.31	10.67^{a}±1.15	9.33^{b}±2.31	8.20c±1.00	8.00^{c}±1.00	8.00^{c}±1.00	6.00^{c}±0.00	0.00
Penicillium chrysogenum	11.33±1.53	10.33±2.52	9.33±1.15	8.67±1.53	8.34±3.06	8.0±2.00	7.00±1.00	0.00
Saccharomyces cerevisiae	11.0±1.0	11.0±1.73	10.0±0.00	9.33±2.08	9.01±1.53	8.00±1.00	6.33±0.58	0.00

Similar letter indicate means that the values not significantly different from each other
Values in Mean ± SEM
Note:
P<0.01, P<0.001 - Highly Significant
P>0.05 - Not Significant

Table 2a: Effect of 28 days daily administration of the aqueous extract of the root of *Z. zanthozyloides* on haematology of wistar rats

Parameters	Concentration (mg/kg)			
	500	1000	2500	Control
RBC	7.45 [a] ± 0.61	125.3 [b] ± 28.62	6.06 [a] ± 0.75	7.12 [a] ± 0.88
PCV	42.50 [a] ± 3.06	43.83 [a] ± 3.21	39.29 [b] ± 4.31	42.98 [a] ± 4.03
Platelets	595.3 [a] ± 193.9	542.4 [b] ± 275.7	661.8 [c] ± 168.9	603.0 [a] ± 156.8

Similar letter indicate means that the values not significantly different from each other
Note:
Values in Mean ± SD
P>0.05 - Not Significant

Table 2b: Effect of 1 week daily administration of the aqueous extract of the root of *Z. zanthozyloides* on haematology of wistar rats

Parameters	Concentration (mg/kg)			
	500	1000	2500	Control
RBC	6.37 [a] ± 0.39	6.32 [a] ± 0.59	5.46 [b] ± 0.54	5.70 [b] ± 0.44
PCV	35.98 [b] ± 6.10	42.18 [a] ± 1.11	35.75 [b] ± 4.14	40.31 [a] ± 1.59
Platelets	315.4 [c] ± 106.7	442.8 [b] ± 104.5	493.5 [b] ± 73.64	612.4 [a] ± 107.1

Similar letter indicate means that the values not significantly different from each other
Values in Mean ± SD
P<0.001; P<0.01 - Highly Significant

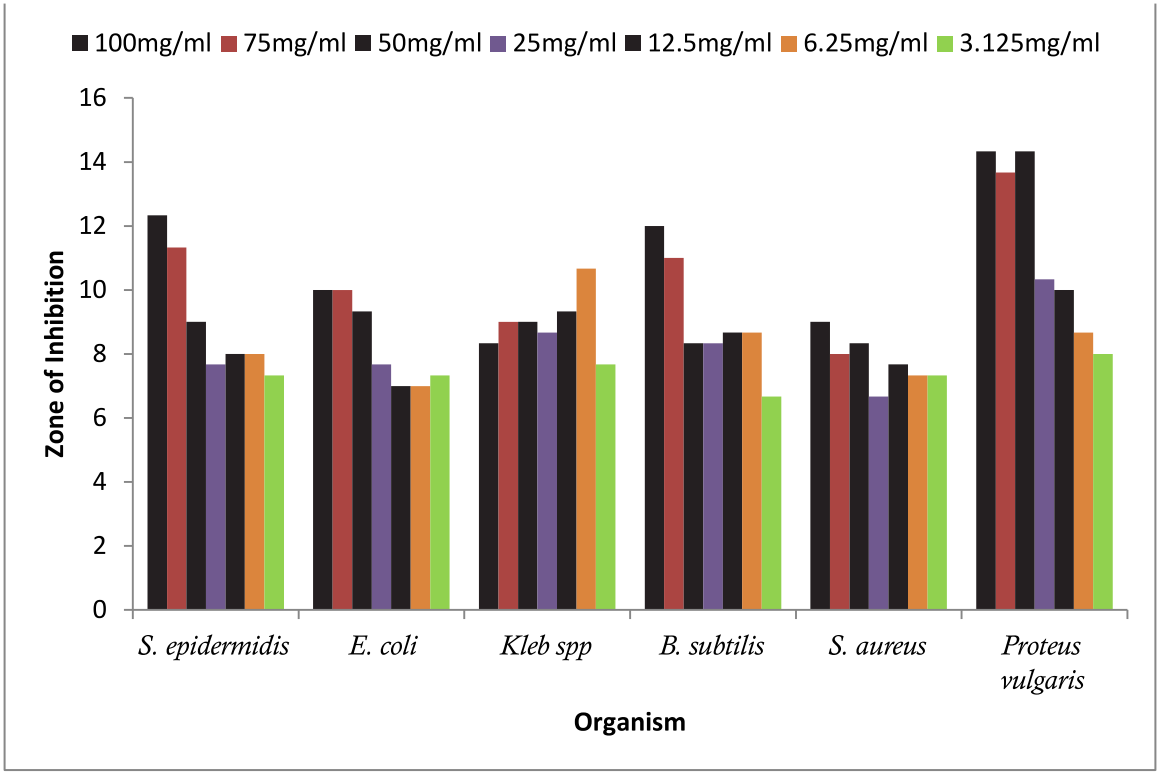

Figure 1a: Zone of inhibition of *Zanthozylum zanthozyloides* extracts on Test organisms

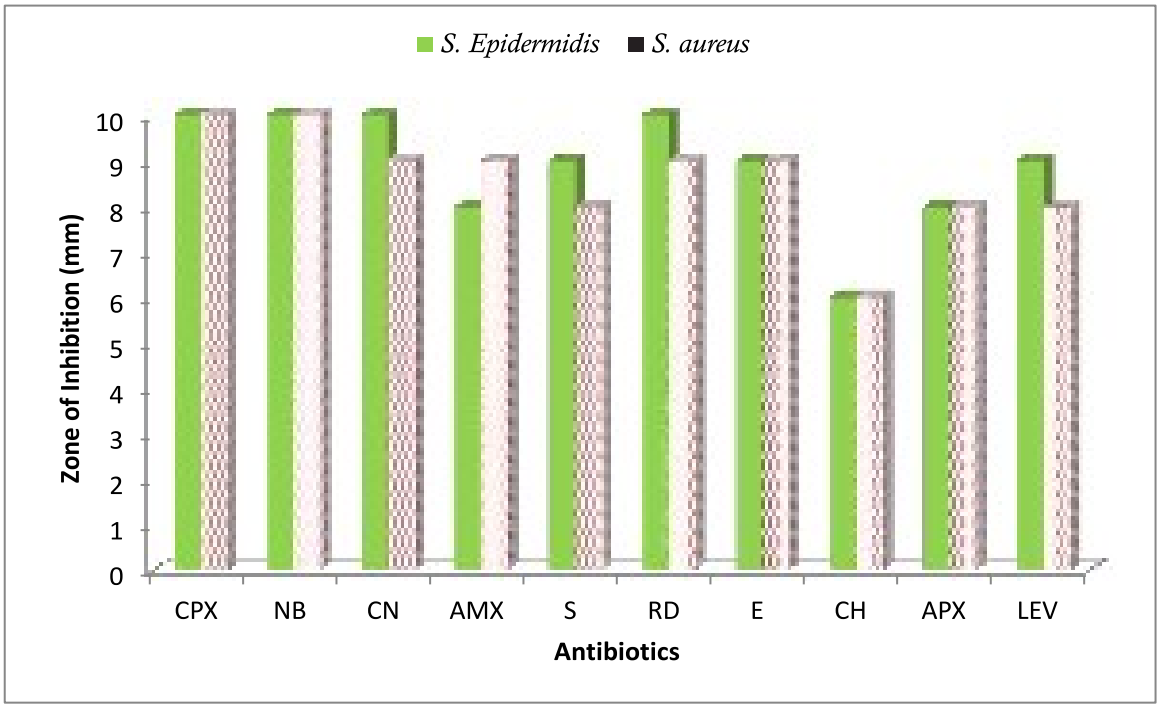

Figure 1b: The zone of inhibition of some commercial Antibiotics on test organism (Gram positive)

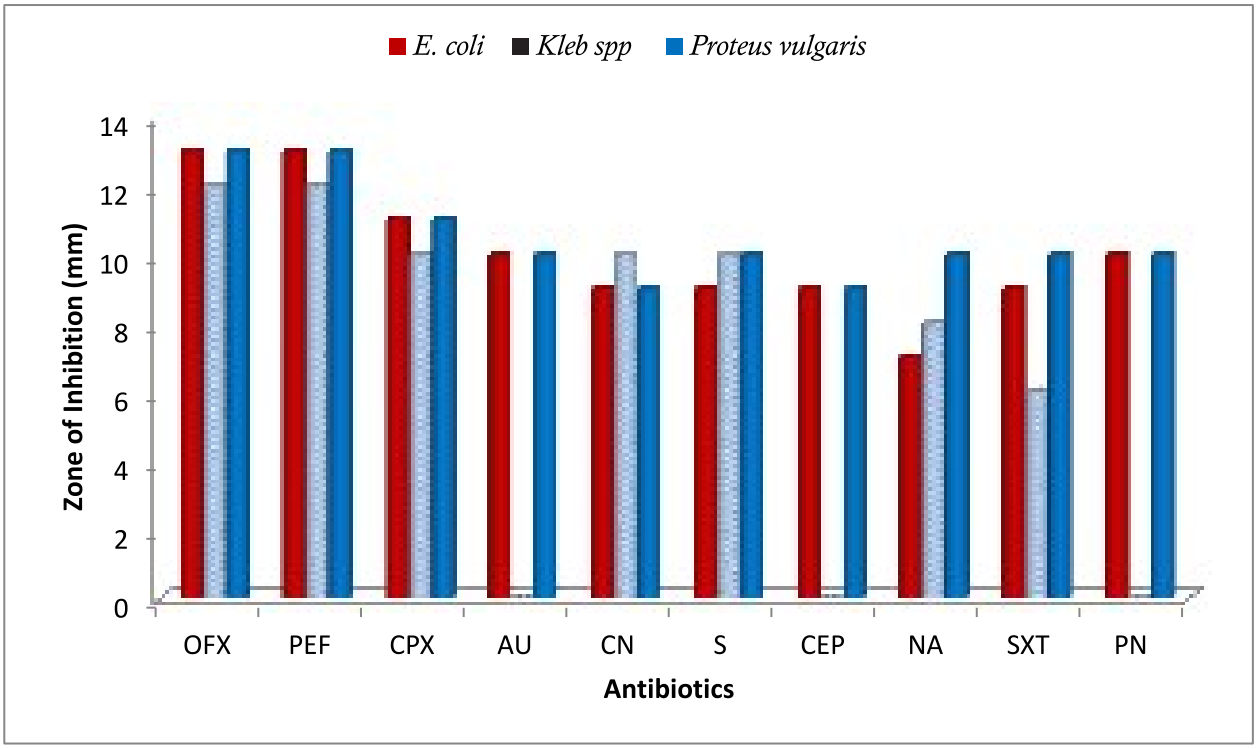

Figure 1c: The zone of inhibition of some commercial Antibiotics on test organism (Gram negative)

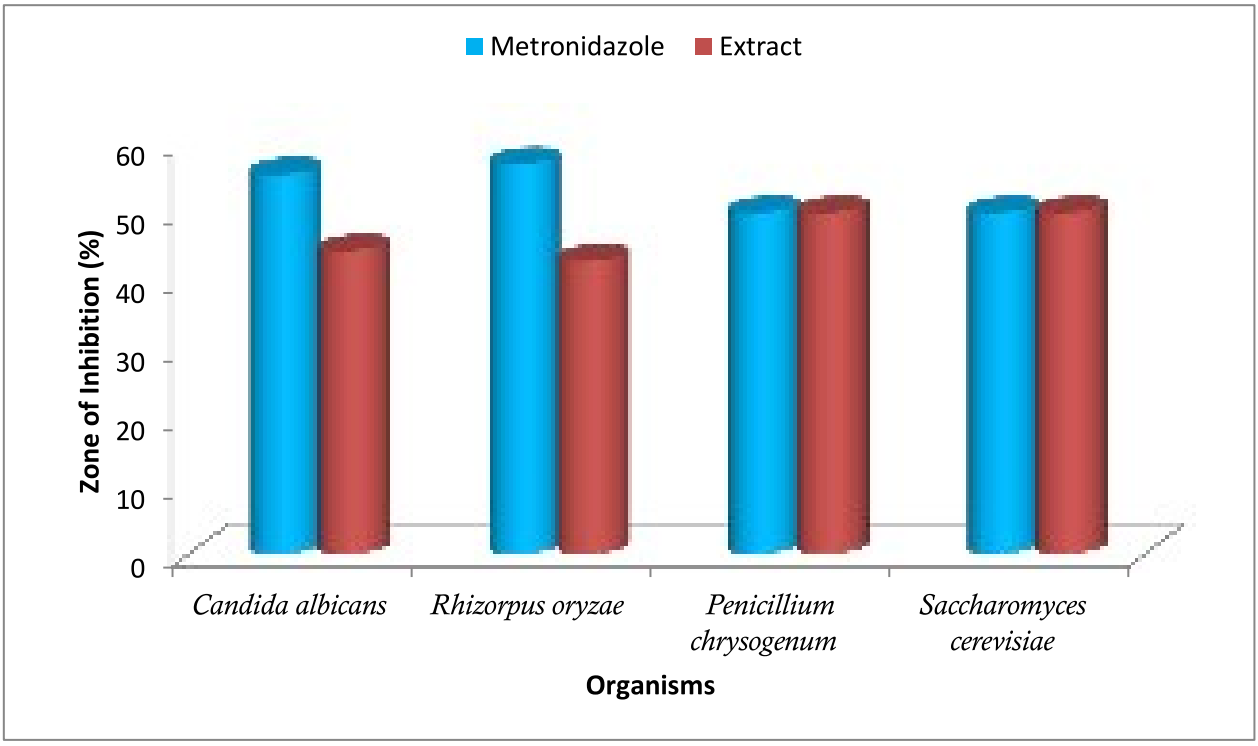

Figure 1d: Comparative zone of inhibition of *Z. zanthozyloides* extracts and Metronidazole on Test fungal isolates

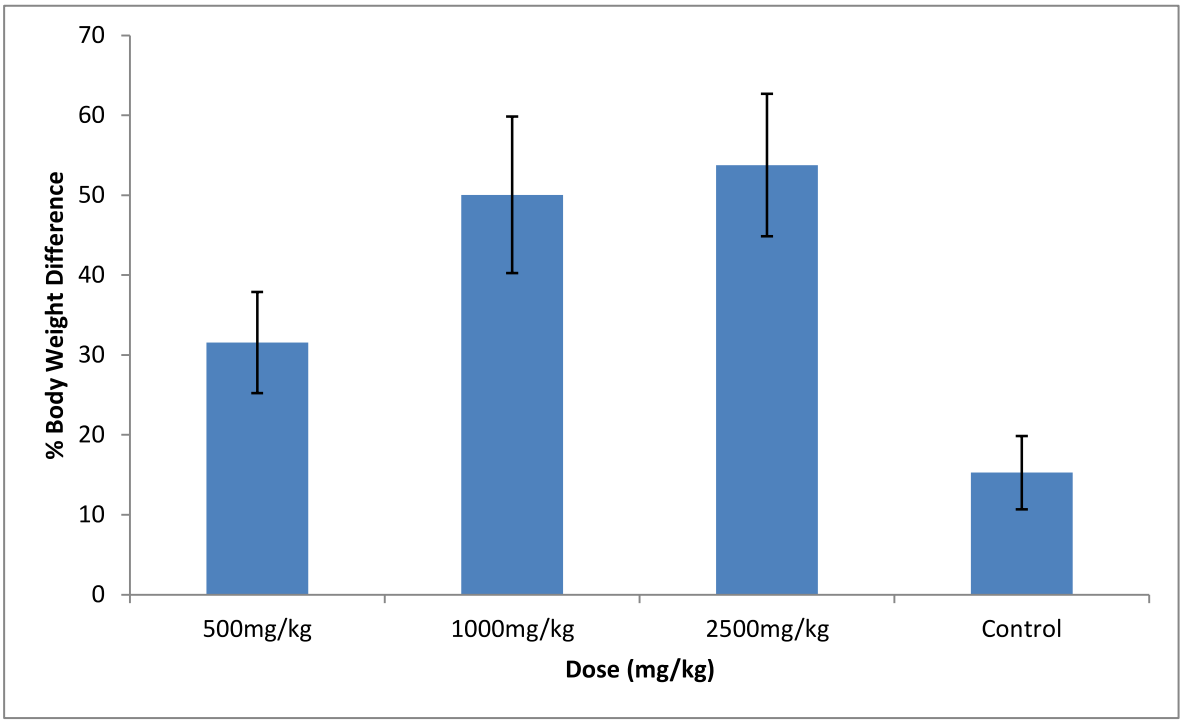

Figure 2a: Weight change in total body weight against dose for sub-chronic toxicity for 28 days administration

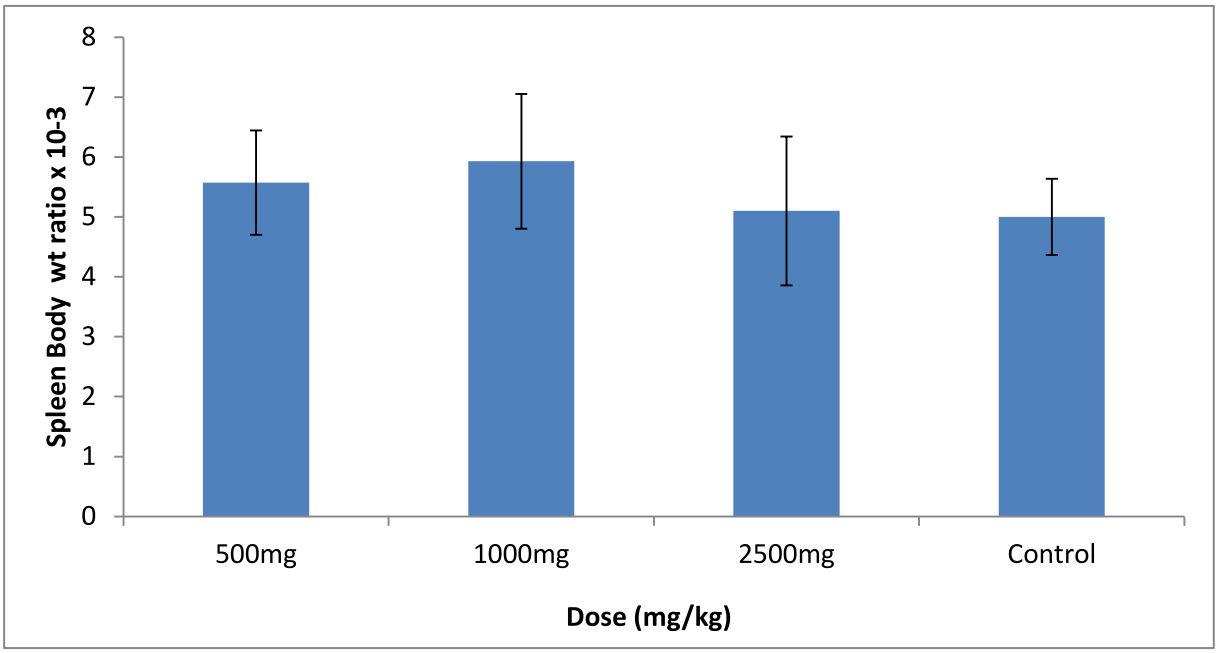

Figure 2b: Weight change in Spleen against dose for sub-chronic toxicity for 28 days administration

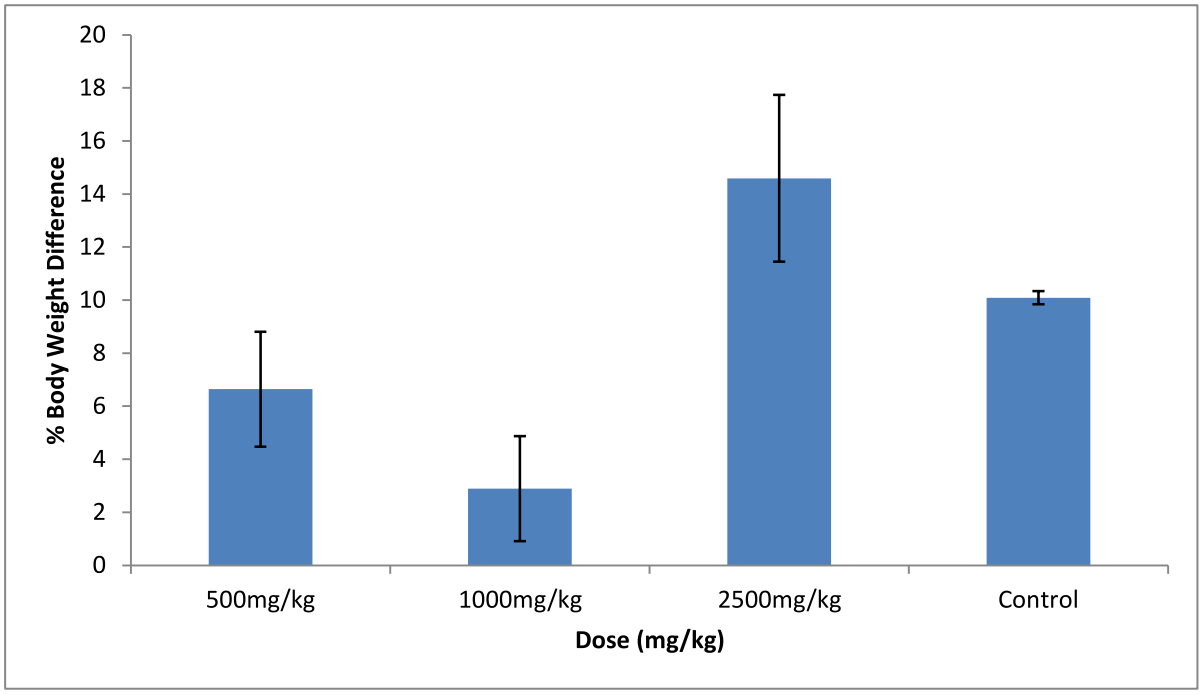

Figure 3a: Weight change in total body weight against dose for sub-acute toxicity for 7 days administration

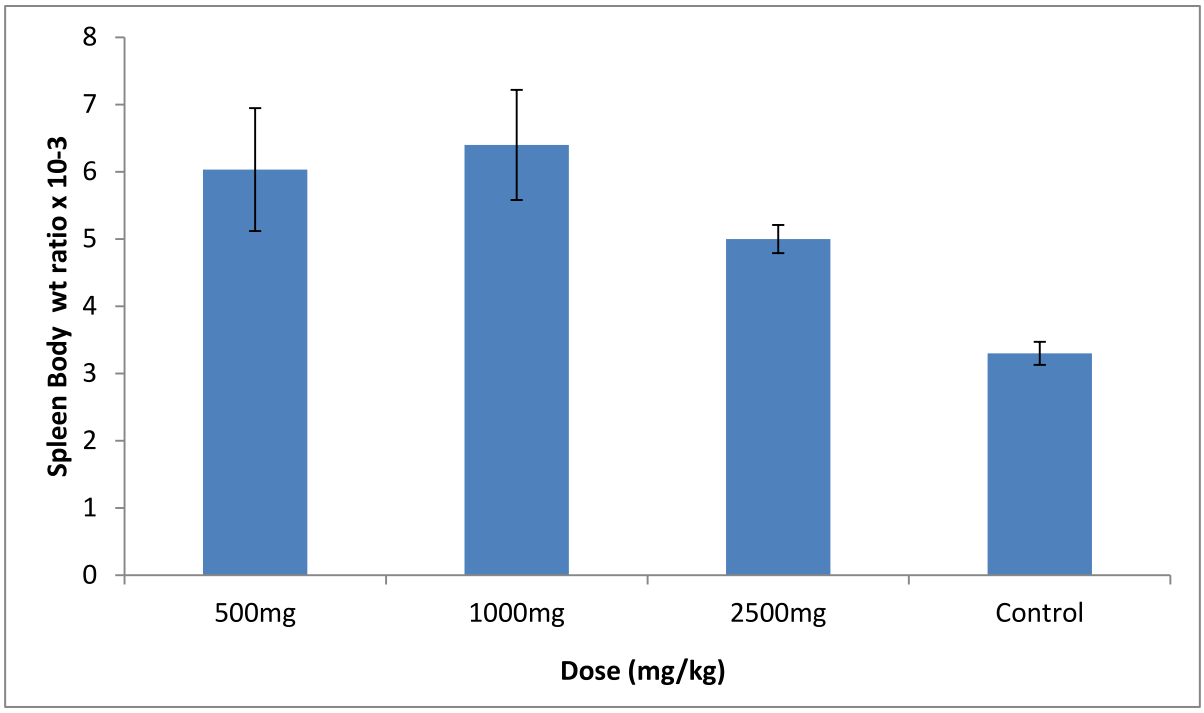

Figure 3b: Weight change in Spleen against dose for sub-acute toxicity for 7 days administration

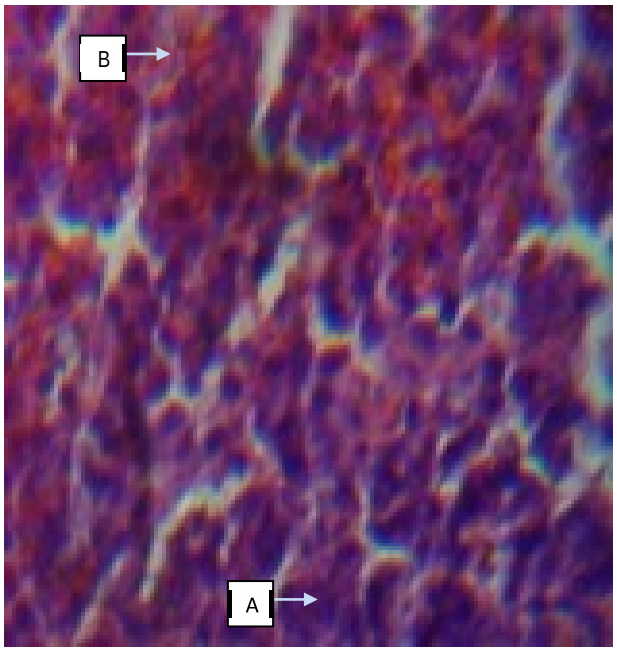

Fig 4a: Control: Rat Spleen showing white pulp A, surrounded by red pulp B (H&E x 400)

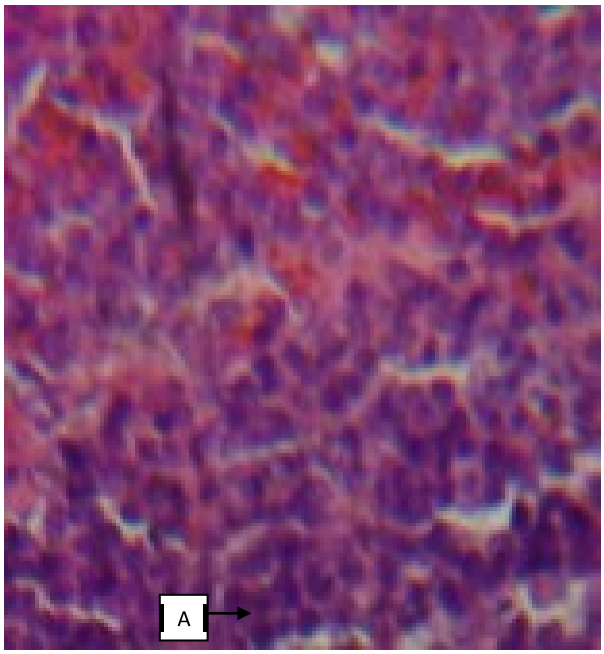

Fig 4b: Rat Spleen treated with 500mg/kg of *Z. zanthozyloides* for 28 days showing mildly activated pulp A (H&E x 400)

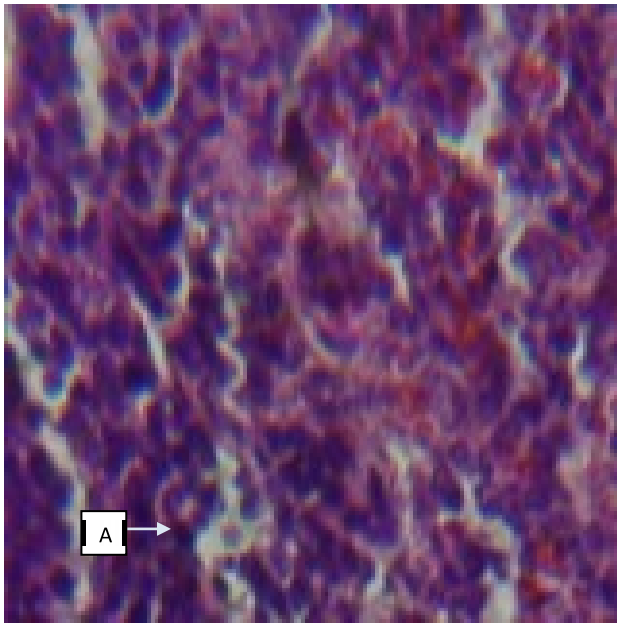

Fig 4c: Rat Spleen treated with 1000mg/kg of *Z. zanthozyloides* for 28 days showing mildly activated pulp A (H&E x 400)

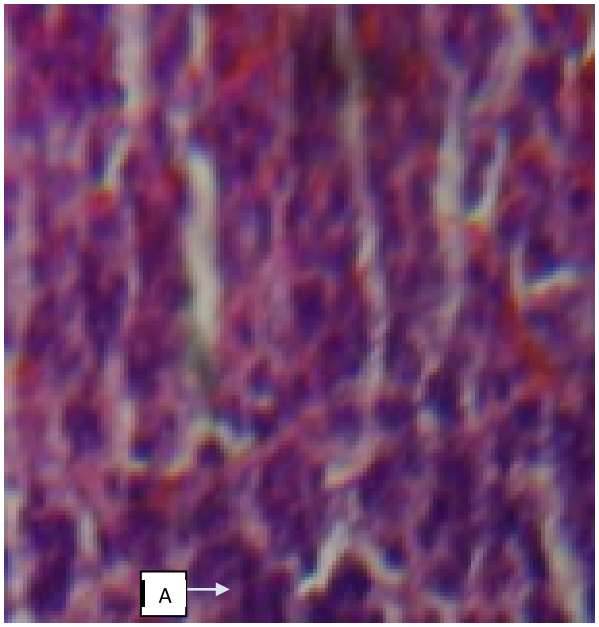

Fig 4d: Rat Spleen treated with 2500mg/kg of *Z. zanthozyloides* for 28 days showing mildly activated pulp A (H&E x 400)

[12-13]. The inhibitory activities may be as a result of the phytochemicals properties of the plant which include cardiac glycosides, alkaloids, saponins, tannins and flavonoids [14]. Although the phytochemistry of *Z. zanthozyloides* was not determined in course of this study but it was reported in literature that the antimicrobial effect of the extract of *Z. zanthozyloides* is as a result of the naturally occurring phytochemicals present in the plant[15].

The aqueous extracts of *Z. zanthozyloides* caused significantly ($P < 0.01$) body weight increase in all the groups of animals that was administered (500, 1000, 2500 mg/kg) *Z. zanthozyloides* root extract for 28 days compared to the control in (fig. 2a), while for 7 days there was an insignificant increase recorded only in 2500mg/kg, but significantly ($P < 0.05$) decreases in the weight of 500mg/kg and 1000mg/kg compared with the control as shown in figure 3a, this supports the work by [16] who reported that *Z. zanthozyloides* extract caused significant increase in body weight of animal models, although no significant difference of body weight ratio of spleen organ was recorded among the animals administered with the graded dose of the extract and that of the control groups (figure 2b and 3b). When treated with graded doses of *Z. zanthozyloides*, the spleen showed mild activation of the white pulp (lymphoid follicles), irrespective of the dose administered (fig. 4a, 4b, 4c and 4d).

Organ weights are widely accepted in the evaluation of test agent-associated toxicities [17]. The Society of Toxicologic Pathology recommends that organ weights be included routinely in toxicity studies for multidose drugs administered in durations from 7 days to 1 year [17]. The aqueous extract of *Z. zanthozyloides* significantly ($P < 0.01$) increase the body weight of the test animals for 28 days administration as against 7 days administration. The increase in body weight may have caused significant changes in the haematological parameters such as RBC, PCV, with significant decrease in PLT (Table 2). The assessments of haematological parameters are used to determine the extent of deleterious effects of the extract on blood of animals [18]. Reduction in RBC and PCV is indication of either the destruction of RBC or their decreased production, which may lead to anaemia [19]. On the contrary an increase in the count of RBC and PCV is suggestive of polycythemia and positive erythopoisis [20-23]. Hence, a significant increase in RBC with no alteration in PCV in *Z. zanthozyloides* treated animals indicates that the extract causes no toxic effect on RBC.

Conclusion

The antimicrobial activities of *Z. zanthozyloides* plant have been previously published by many authors, the results of this study shows a significant ($P < 0.01$, $P < 0.001$) inhibition against *E. coli, P vulgaris, S. epidermidis and R. oryzae* while at $P > 0.05$ inhibited *Klebsiella* spp., *S. aureus, P. chrysogenum, S. cerevisiae* and *C. albicans* at various concentration ranging from 100 to 3.125mg/kg. This study showed that the aqueous extracts of *Z. zanthozyloides* could be used as a broad spectrum antibiotics as it inhibits the growth of both bacteria and fungi organisms, while it was seen to be more effective compared to commercial antibiotics such as; Augmentin, Ceporax and Amplicin antibiotics against *Klebsiella* spp., as the organism was resistant to these commercial antibiotics. The aqueous extract *Z. zanthozyloides* significantly ($P < 0.01$) increased the body weight of the test animals for 28 days

administration, causing increase in the haematological parameter such as RBC, PCV and decrease PLT which were significant at ($P < 0.01$, $P < 0.001$) for 7 days administration. These alterations were regulated by the body system of the test animal to insignificant level ($P > 0.05$) compared to the control groups as shown in 28 days administration, therefore no significant damage was recorded on the spleen organ analysed.

References

1. Adebayo, J. O. and Krettli, A. U. (2011). Potential antimalarials from Nigerian Plants: A review. Journal of Ethnopharmacology 133: 289-302.

2. Adesina, S. K. (2005). The Nigerian *Zanthozylum*: Chemical and Biological Values. African Journal of Traditional Complementary and Alternative medicines 2: 282-301.

3. Seidemann, J. (2005). World Spice Plants: Economic Usage, Botany, Taxonomy. Springer, New Mexico, USA, 592 p.

4. Chase, M. W., Morton, C. M. and Kallunki, J. A. (1999). Phylogenetic Relationships of Rutaceae: a Cladistic Analysis of the Subfamilies Using Evidence from rbcLand atpB Sequence Variation. American Journal of Botany 86: 1191-1199.

5. Sofowora, E. A., Isaac-Sodeye, W. A. and Ogunkoya, L. O. (1995). Isolation and characterization of an antisickling agent from Fagarazanthoxyloides root. Lloydia 38: 169-174.

6. Andersson, C. M., Halberg, A. and Hogberg, T. (1996).Advances in the Development of Pharmaceutical Antioxidants.Advances in Drug Research 28: 65-180.

7. Adesina, S. K. (2005). The Nigerian Zanthozylum: Chemical and Biological Values. African Journal of Traditional Complementary and Alternative medicines2: 282-301.

8. Odebiyi, O. O. and Sofowora, E. A. (1979). Antimicrobial alkaloids from Nigerian chewing stick (Fagarazanthoxyloides). Planta Medicine 36:204-207.

9. Tsuchiya, H., Sato, M., Miyazaki, T., Fujiwara, S., Tanigaki, S., Ohyama, M., Tanaka, T. andIinuma, M. (1996). Comparative study on the antibacterial activity of phytochemical flavones against methicillin-resistant Staphylococcus aureus. Journal ofEthnopharmacology 50:27-34.

10. Olatunji, O. A. (1983). The biology of Zanthoxylum Linn (Rutaceae) in Nigeria, in "Anti-infective agents of higher plants origin." Proceedings of the Fifth International Symposium on Medical Plants, pp. 56-59

11. Da Silva, S. L., Figueredo, P. M. S. and Yano, T. (2006).Antibacterial and Antifungal Activities of Volatile Oils from ZanthozylumrhoifoliumLam. Leaves. Pharmaceutical Biology 44: 657-659.

12. Mbaze, L. M., Poumale, H. M. P., Wansi, J. D., Lado, J. A., Khan, S. N., Iqbal, M. C., Ngadjui, B. T. and Laatsch, H. (2007). Glucosidase inhibitory pentacyclictriterpenes from the stem bark of Fagaratessmannii(Rutaceae). Phytochemistry 68:591-595.

13. Ynalvez, R. A., Cardenas, C., Addo, J. K. Adukpo, G. E., Dadson, B. A. and Addo-Mensah, A. (2012). Evaluation of the antimicrobial activity of Zanthoxylumzanthoxyloides root bark extracts. Research Journal of Medical Plant 63:149-159.

14. Adegbolagun, O. M. and Olukemi, O. O. (2010).Effect of light irradiation on the antimicrobial activity of Zanthoxylumzanthoxyloides (Lam) methanolic extract.African Journal of Pharmacy and Pharmacology 4(4): 145-150.

15. Itemire, O. A., Ogbimi, O. A. and Idu, M. (2013). Phytochemistry and antimicrobial activity of Zanthoxylum zanthoxyloides root used as chewing stick in Nigeria. Journal of Phytopharmacology 2(6):1-7.

16. Nwozo, S.O., Orojobi, B. F. and Adaramoye, O.A. (2011).Hypolipidemic and antioxidant

potentials of Xylopiaaethiopica seed extract in hypercholesterolemic rats. Journal of Medicinal Food 14(1-2):114-9.

17. Sellers, R. S., Morton, D., Michael, B., Roome, N. and Johnson, J. K. (2007). Society of toxicologic pathology position paper: Organ weight recommendations for toxicology studies. Toxicology and Pathology 35:751-755.

18. Mishra, N. and Tandon, V. L. (2012). Haematological effect of aqueous extract of ornamental plants in male Swiss albino mice. Veterinary World 5(1):19.23.

19. Adedapo, A. A., Abatan, M. O. and Olorunsogo, O. O. (2007). Effect of some plants of the spurge family on haematological and biochemical parameters in rats. Veterinarski Archives 77:29-38.

20. Iranloye, B. O. (2002). Effect of chronic garlic feeding on some haematological paramenters. Africa Journal of Biomedical Research 5:81-82.

21. Mansi, K. and Lahham, J. (2008). Effect of Artemisia sieberi Besser (A/ herba-alba) on heart rate and some haematological values in normal and alloxan induced diabetic rat. Journal of Basic and Applied Sciences 4:57-62

22. Kuppast, I., Vasudeva, J., Nayak, P., Ravi, M. C. and Biradar, S. S. (2009). Studies on the haematological effect of the extracts of Cordiadichotoma Forst. F. Fruits. Research Journal of Pharmacology and Pharmacodynamics 1:117-119.

23. Okpuzor, J., Ogbunugafor, H. A. and Kareem, G. K. (2009). Hepatoprotective and hematologic effects of fractions of Globinetula braunii in normal albino rats. EXCIL Journal 8:182-189.

24. Eloff, J.N. (1998). A sensitive and quick micro-plate Method to determine the minimal inhibitory concentration of plants extracts for bacteria. Panta Medica, 64:711-713.