



International Journal of Traditional and Complementary Medicine
(ISSN:2474-1361)



In Vivo effects of dosage of leaf, bark and root extracts of *V. paradoxa* on diarrhoea-induced albino rats

M. B. FBALANA^{1*}, M. O. BANKOLE² and A. M. OMEMU³

^{1*}Department of Biological Sciences, AL- Hikmah University Ilorin, P.M.B 1601, Nigeria.

²Department of Microbiology, Federal University of Agriculture, Abeokuta (FUNAAB), P.M.B 2240, Nigeria.

³Department of Food Service and Tourism, Federal University of Agriculture, Abeokuta (FUNAAB), P.M.B 2240, Nigeria.

ABSTRACT

In vivo study of different dosages (50mg/kg and 100mg/kg) of various solvent extracts (water, methanol, omidun and sterile omidun) of Leaf, Root and Bark of *Vitellaria paradoxa* were examined on male and female albino rats induced with diarrhoea-causing microorganisms (*Enterohaemorrhagic Escherichia coli* (EHEC ATCC 43889), *Enteropathogenic Escherichia coli* (EPEC ATCC 43887), *Salmonella typhi*, and *Escherichia coli* ATCC 25922). The rats were randomly assigned into four groups (A-D), group A rats infected with *Enteropathogenic Escherichia coli* (EPEC ATCC 43887) and later treated with ciprofloxacin, group B rats were infected with EPEC only, group C rats were infected with EPEC and treated with 50mg/kg concentration of methanol extract of leaf of *V. paradoxa*, while group D rats were not infected nor treated. The procedure was repeated for other test diarrhoea-causing microorganisms and 100mg/kg concentrations of all the solvent extracts of root and bark respectively. The rats were observed hourly for six hours for the presence or absence of diarrhoea. Haematological studies was conducted on the blood samples of the rats. All extracts of *V. paradoxa* have anti-diarrhoea effect at both concentrations tested against all the tested diarrhoeagenic bacteria. Although the type of diarrhoea-inducing microorganisms did not significantly affect the activity of the plant parts and 50mg/ml concentration of water extract exhibited more significant reduction in diarrhoea symptoms than 100mg/ml concentration of other solvent extracts. Bark and leaf extracts of *V. paradoxa* was also more effective at reducing symptoms of diarrhoea in the experimental rats than root extracts. Levels of the major blood parameters (White Blood Cell, Red Blood Cell, Haemoglobin and Packed Cell Volume) were not significantly different ($P > 0.05$) among the rats treated with the plant parts but slightly higher in rats treated with bark, followed by root and leaves.

Keywords: Omidun, *Vitellaria paradoxa*, Diarrhoeagenic

*Correspondence to Author:

M. B. FBALANA

Department of Biological Sciences,
AL- Hikmah University Ilorin, P.M.B
1601, Nigeria

How to cite this article:

M. B. FBALANA, M. O. BANKOLE and A. M. OMEMU. In Vivo effects of dosage of leaf, bark and root extracts of *V. paradoxa* on diarrhoea-induced albino rats. International Journal of Traditional and Complementary Medicine 2018, 3:8.

 eSciPub
eSciPub LLC, Houston, TX USA.
Website: <http://escipub.com/>

Introduction

Medicinal plants would be the best source to obtain a variety of drugs probably due to presence of various complex chemical substance of different composition, which are found as secondary plant metabolites in one or more parts of these plants (Kumar *et al.*, 2010). This drives the need to screen medicinal plants for novel bioactive compounds as plant based drugs are biodegradable, safe and have fewer side effects (Govindaraji *et al.*, 2008). There are many forms in which herbs can be administered, the most common of which is in the form of a liquid that is drunk by the patient, either a tisane or a (possibly diluted) plant extract (Saad *et al.*, 2011). Many researchers reported influence of different extraction solvents and techniques on the content of natural antioxidants in extracts (Michelis *et al.*, 2012).

Vitellaria paradoxa (Sapotaceae) is a plant that is locally abundant in Nigeria in the derived Savannah zones (12°00'N, 8°31'E) particularly near towns and villages. It is rich in oil and replaces the oil palm as a source of edible oil in Northern Nigeria (Lamien *et al.*, 2007). In Africa where the species occurs, the fruit pulp can be eaten by both humans and manufacturing body care products as well as pharmaceutical and confectionery industries (Awolaye, 1995; Kelly *et al.*, 2004). The seed fat is used for cooking, in lighting of lamps, in soap and pomade preparations as well as for medicinal purposes (ICRA, 2000; Popoola and Tee, 2001). Other plant parts have been credited with various medicinal uses (Collinson and Zewdie-Bosuener, 1999).

Collinson and Zewdie-Bosuener (1999) and Ndukwe *et al.*, (2001) variously reported works on this plant which focused essentially on the fruit, kernel, seed and the fat from the seed. Study conducted by Abubakar *et al.* (2013) also revealed that methanol stem bark extract *Vitellaria paradoxa* may possess anti diarrhoeal property. Phytochemical screening of the stem bark of *V. paradoxa* revealed the presence of carbohydrates, alkaloids, saponins, tanins and

cardiac glycosided (Collinson and Zewdie-Bosuener, 1999).

Hence, this study seeks to further investigate the traditional use of the liquid obtained from soaking the bark, leaves and roots of *V. paradoxa* in water and other solvents (methanol, non-sterile *omidun* and sterile *omidun*) to treat ailments associated with diarrhoea by specifically checking whether each of the solution has protective effects on some bacteria that cause diarrhoea and to know whether the variety of solvents used in extracting the plant parts have any effect on the efficacy of different parts of *Vitellaria paradoxa* through *In vivo* assay.

Materials and Methods

Test microorganisms

The diarrhoeagenic bacteria used for this study were clinical isolate (*Salmonella typhi*) obtained from Sacred Heart Hospital Lantoro, Abeokuta Nigeria and typed culture Enteropathogenic *Escherichia coli* (EPEC ATCC 43887), Enterohaemorrhagic *Escherichia coli* (EHEC ATCC 43889) *Escherichia coli* ATCC 25922 obtained from National institute of medical research, Yaba Lagos Nigeria. The organisms were subjected to cultural and morphological identification and finally biochemical characterization using protocols described by Cheesbrough (2002). Pure cultures of the isolates were maintained on appropriate media for future use

Experimental Animal

The experimental animal used in this study were albino rats (weighing between 180 – 220g), which were purchased from the Animal House University of Ibadan Nigeria. The rats were acclimatized to standard laboratory conditions (temperature 24 ± 1°C and a 12 hours photoperiod), hygienically fed twice daily with standard commercial feeds (Vital Feeds, Nigeria) and sterile distilled water *ad libitum* for one week before the commencement of the experiment.

Ethical Considerations

Albino rats used for this research were appropriately selected. Rats were kept in Laboratory cages and maintained according to the NIH guidelines of care and use of laboratory Animals published by Saha *et al.* (2001) while standards of the animal care and administration met those required by applicable international laws and regulations. Painful procedures were performed under anesthesia to avoid distress and pain (Naderi *et al.*, 2012) to the albino rats.

Collection and Preparation of Plant Materials

The plant materials used for this study were leaf, bark and root of *Vitellaria paradoxa* (Shea butter tree). The plant materials were collected from Onipako village in Ilorin, Kwara State of Nigeria, confirmed by local farmers and further identified (Identification Number: UAHA NO. 015/001) and authenticated in the Herbarium Laboratory of the Department of Forestry and Wildlife Management, Federal University of Agriculture, Abeokuta. The collected Plant materials were washed with sterile water and dried under shade; they were reduced into small pieces with a surface-sterilized scalpel before milling with a separate electric blender (Model Marlex) for each plant part.

Extraction Procedures

A quantity (150 g) of the fine powder of the leaf was each weighed into four 1000 mL capacity conical flask and 500 ml methanol, sterile distilled water, sterile *omidun* and non-sterile *omidun* was added to powder in a conical flask respectively. This procedure was repeated for the root and bark samples to give a total of twelve (12) 1000ml capacity conical flasks. Each was allowed to stand for 48hours with constant shaking at regular intervals to facilitate extraction (Otimenyin *et al.*, 2008). The percolates were then filtered and the resulting volume on filtration was reduced to dryness with a Rotary evaporator (RE 100 – Pro) at $45 \pm 10^{\circ}\text{C}$. The extracts were then collected, weighed, packed in sterile air tight containers and labeled. They were kept in the refrigerator at 4°C until needed for analysis.

In Vivo antimicrobial assay

The albino rats were weighed prior to the experiment and denied access to food for 24 hours before commencement of the experimental procedures, but were allowed free access to sterile distilled water. The rats were randomly divided into 5 groups A-E of 5 rats each and fed as follows; Group A ciprofloxacin and organism, group B organism only, group C organism and different extract, group D sterile distilled water extract only and group E received nothing with each rat in group A-C orally given 0.5ml of 18 hour broth culture of the test organisms to induce diarrhoea.

Each rat received (oral) 0.5ml of 18-hr broth culture of the test organisms to induce diarrhoea respectively. Thirty minutes later, all groups A-D were intraperitoneally treated as follows: Group A received 2mls of commercially prepared ciprofloxacin, two subgroups of C received 2mls of 50mg/kg and 100mg/kg of different extracts of the plant parts respectively, group D received 2mls sterile distilled water extracts of the plant parts only, while group B. The treatment was repeated for each of the 5 test organisms. The treated rats were then placed in separate cages over clean white paper and observed for the presence of diarrhoea every one hour for six hours for the onset of diarrhoea and number of diarrhoea episodes. Absence or reduction in number of loose stool was recorded as a protection from diarrhoea (Bibitha *et al.*, 2002).

Results

Effect of solvent extracts of leaf, bark and root of *V. paradoxa* on diarrhoeal-induced rats

Figure (1) below shows the effect of treatment with different concentrations of various solvent extracts of leaf, root and bark of *V. paradoxa* on total number of rats with diarrhoea from diarrhoeagenic bacteria. Treatments with 50mg/mL concentrations of *omidun* extracts of the leaf, root and bark of *V. paradoxa* revealed that seven out of the rats treated with leaf extracts showed diarrhoea symptom, eight out of

the rats treated with root extracts showed diarrhoea symptom and four out of the rats treated with bark extracts showed diarrhoea symptom. Also, treatments with 50mg/mL concentrations of sterile *omidun* extracts of the leaf, root and bark of *V. paradoxa* revealed that four out of the rats treated with leaf extracts showed diarrhoea symptom, five out of the rats treated with root extracts showed diarrhoea symptom and six out of the rats treated with bark extracts showed diarrhoea symptom.

But with 50mg/mL concentrations of methanol extracts of the leaf, root and bark of *V. paradoxa* revealed that four out of the rats treated with leaf extracts showed diarrhoea symptom, two out of the rats treated with root extracts showed diarrhoea symptom and one out of the rats treated with bark extracts showed diarrhoea symptom. Treatments with 50mg/mL concentrations of water extracts of the leaf, root and bark of *V. paradoxa* revealed that one out of the rats treated with leaf extracts showed diarrhoea symptom, three out of the rats treated with root extracts showed diarrhoea symptom and two out of the rats treated with bark extracts showed diarrhoea symptom.

Also, treatments with 100mg/mL concentrations of *omidun* extracts of the leaf, root and bark of *V. paradoxa* revealed that seven out of the rats treated with leaf extracts showed diarrhoea symptom, nine out of the rats treated with root extracts showed diarrhoea symptom and six out of the rats treated with bark extracts showed diarrhoea symptom. Treatments with 100mg/mL concentrations of sterile *omidun* extracts of the leaf, root and bark of *V. paradoxa* revealed that two out of the rats treated with leaf extracts showed diarrhoea symptom, seven out of the rats treated with root extracts showed diarrhoea symptom and six out of the rats treated with bark extracts showed diarrhoea symptom.

However, treatment with 100mg/mL concentrations of methanol extracts of the leaf, root and bark of *V. paradoxa* revealed that five out of the rats treated with leaf extracts showed diarrhoea symptom, three out of the rats treated

with root extracts showed diarrhoea symptom and seven out of the rats treated with bark extracts showed diarrhoea symptom. Treatments with 100mg/mL concentrations of water extracts of the leaf, root and bark of *V. paradoxa* revealed that four out of the rats treated with leaf extracts showed diarrhoea symptom, eight out of the rats treated with root extracts showed diarrhoea symptom and four out of the rats treated with bark extracts showed diarrhoea symptom.

General Percentage Protection of Rats from Diarrhoea Inducing Bacteria Tested with Omidun extracts of leaf, bark and root of *V. paradoxa*

Figure 2 shows the percentage protection from diarrhoea in diarrhoea induced rats treated with *omidun* extracts of leaf, bark and root of *V. paradoxa*. The result obtained from treatments with different concentrations (50mg/mL and 100mg/mL concentrations) of these extracts revealed that the various extracts of *omidun* conferred protection on rats that had been induced with some diarrhoea microorganisms. The leaf extract conferred the highest protection from diarrhoea (38%), this was followed by the bark extract (33%) and the least protection (29%) was conferred by the root extract.

General Percentage Protection of Rats from diarrhoea Inducing Bacteria Tested with Methanol extracts of leaf, bark and root of *V. paradoxa*

Figure 3 shows the percentage protection from diarrhoea in diarrhoea induced rats treated with methanol extracts of leaf, bark and root of *V. paradoxa*. The result obtained from treatments with different concentrations (50mg/mL and 100mg/mL concentrations) of these extracts revealed that the various extracts of methanol conferred protection on rats that had been induced with some diarrhoea causing microorganisms. The root extract of methanol conferred the highest protection from diarrhoea (35%), this was followed by the bark extract of

methanol (33%) and the least protection (32%) was conferred by the leaf extract of methanol.

General Percentage Protection of Rats from diarrhoea Inducing Bacteria Tested with Sterile Omidun extracts of leaf, bark and root of *V. paradoxa*

Figure 4 shows the percentage protection from diarrhoea in diarrhoea induced rats treated with sterile amidun extracts of leaf, bark and root of *V. paradoxa*. The result obtained from treatments with different concentrations (50mg/mL and 100mg/mL concentrations) of these extracts revealed that the various extracts of sterile amidun conferred percentage protection on rats that had been induced with some diarrhoea causing microorganisms. The leaf extract of sterile amidun conferred the highest protection from diarrhoea (40%), this was followed by the bark extract of sterile amidun (32%) and the least protection (28%) was conferred by the root extract of sterile amidun.

General Percentage Protection of Rats from diarrhoea Inducing Bacteria Tested with Water extracts of leaf, bark and root of *V. paradoxa*

Figure 5 shows the percentage protection from diarrhoea in diarrhoea induced rats treated with sterile amidun extracts of leaf, bark and root of *V. paradoxa*. The result obtained from treatments with different concentrations (50mg and 100mg/mL concentrations) of these extracts revealed that the various extracts of sterile

omidun conferred percentage protection on rats that had been induced with some diarrhoea causing microorganisms. However, the leaf extract of sterile amidun conferred the highest protection from diarrhoea (35%), this was followed by the root extract of sterile amidun (33%) and the least protection (32%) was conferred by the bark extract of sterile amidun.

Summary of effects of root, leaf and bark of *V. paradoxa* on major blood Parameters of Experimental Albino Rats

Summarily, there was no significant difference ($P > 0.05$) in the effect of root, leaf and bark of *V. paradoxa* on the major blood parameters of the experimental Albino Rats. Although, level of WBC count was higher in rats treated with bark extracts (5.388), followed by those treated with root extracts (5.289) but was slightly lower in rats treated with leaf extracts (4.808). However, levels of RBC count was significantly different ($P < 0.05$) in rats treated with leaf, root and bark of *V. paradoxa*. RBC count in rats treated with bark extracts was 8.758, the count in rats treated with root extracts was 7.693 and lowest count (6.517) in rats treated with leaf extracts. Level of PCV was significantly ($P < 0.05$) high (569.517) in rats treated with bark extracts, followed by those treated with root and the leaf extracts (319.924% and 316.282% respectively). However, there was significant difference ($P < 0.05$) in the levels of all other parameters (MCV, MCHC, MCH and HGB) in rats treated with leaf, root and bark extracts and commercial antibiotics

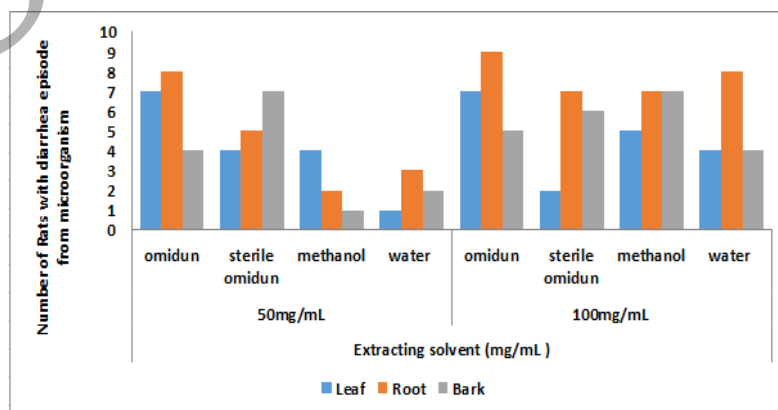


FIGURE 1: Effect of solvent extracts of leaf, bark and root of *V. paradoxa* on diarrhoeal-induced rats.

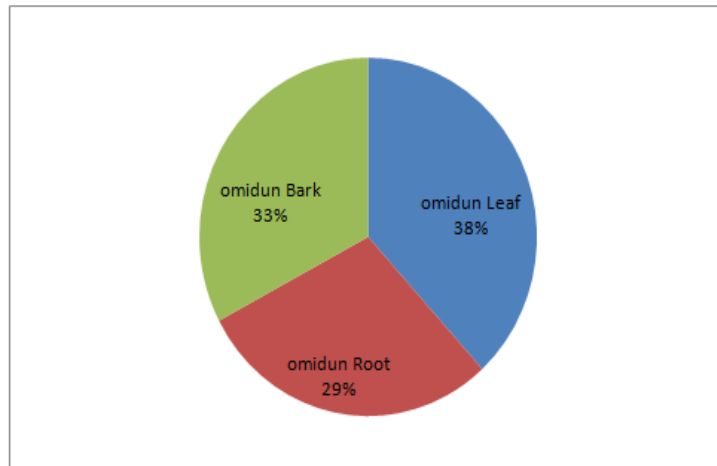


Figure 2: General Percentage Protection of Rats from Diarrhoea Inducing Bacteria Tested with Omidun extracts of leaf, bark and root of *V. paradoxa*

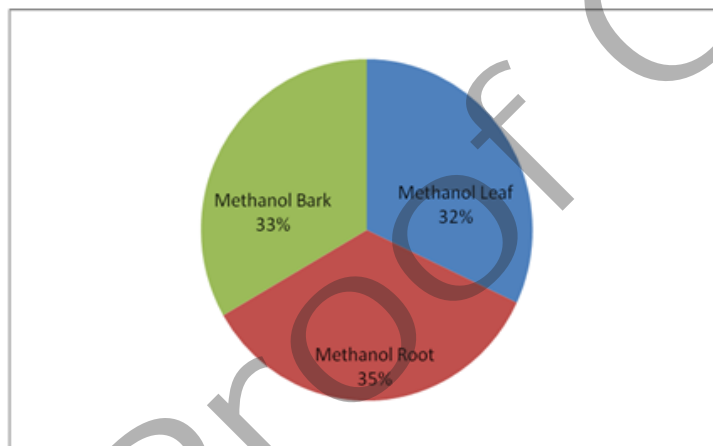


Figure 2: General Percentage Protection of Rats from diarrhoea Inducing Bacteria Tested with Methanol extracts of leaf, bark and root of *V. paradoxa*

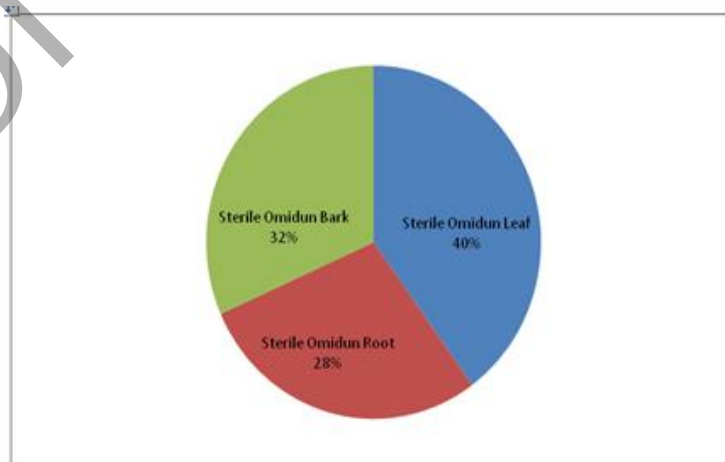


Figure 3: General Percentage Protection of Rats from diarrhoea Inducing Bacteria Tested with Sterile Omidun extracts of leaf, bark and root of *V. paradoxa*

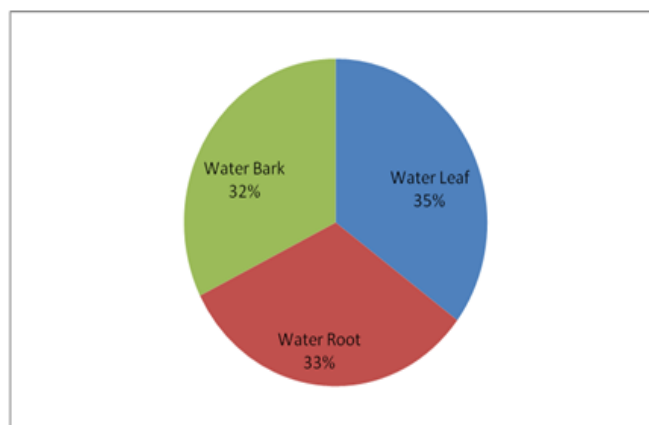


Figure 4: General Percentage Protection of Rats from diarrhoea Inducing Bacteria Tested with Water extracts of leaf, bark and root of *V. paradoxa*

Table 1: Summary of effects of root, leaf and bark of *V. paradoxa* on major blood Parameters of Experimental Albino Rats

Blood Parameters	Root	Leaf	Bark
WBC	5.289 ^a	4.808 ^a	5.388 ^a
RBC	7.693 ^b	6.517 ^c	8.758 ^a
HGB	140.925 ^a	137.325 ^a	144.844 ^a
PCV	319.942 ^b	316.282 ^b	569.517 ^a

Values with different superscript on the same row are significantly different ($p < 0.05$)

Discussion

The bark extracts of *V. paradoxa* were found most inhibitory, followed by the leaf and finally the root (Figure 1). Also, water extracts reduced diarrhoea episodes more than other solvents of extraction. More of the rats treated with 100mg/ml extract of the plant parts showed diarrhoea symptoms more than those treated with 50mg/ml extracts. This variation in activities of leaf, root and bark extracts of *V. paradoxa* showed that bioactive components are present in varying degrees in the different plant parts and that different plant parts had varied degrees of solubility in various solvents used for their extraction. This result further support the report of Bibitha *et al.* (2002) that variations exist in the antibacterial activities of different plant extracts. On the other hand, the observed variations in the

potency of these plant parts were also in conformity with the reports of Duke (1992) and Yusha'u *et al.* (2008) that antibacterial activity may vary from one plant part to another.

In this study, it was observed that methanol and water (Figure 3 and 5) extracts reduced the occurrence of loose stool than other solvent extracts. This difference is attributed to the solubility of the active component in different solvents Karou *et al.* (2007). This may also mean that methanol and water extracted more of active constituents of the leaf, bark and root of *V. paradoxa* which are reported to be antimicrobial in activity (Okwu and Josiah, 2006). Akinpelu *et al.* (2011) also reported the bioactivity of the methanolic crude leaf extract of *D. guineense* on some bacteria.

The antimicrobial activities of these plant extracts may not be due to the action of a single active compound, but the synergistic effect of several compounds that are in minor proportion in the plant parts (Davinco *et al.*, 2007). Furthermore, the observed antimicrobial property of *V. paradoxa* corroborates its use in traditional medicine. The antibacterial activities of these plant extracts against the tested organisms may be due to the presence of bioactive components present in the extract. Ebanu *et al.* (Ebanu *et al.*, 1999) states that antimicrobial activity in plants results from the bioactive component such as alkaloids, saponins, tannins, anthraquinones, steroids, flavonoids present in the plants.

Major blood parameters of the experimental rats reduced but not significantly ($p > 0.05$) among blood samples of rats treated with the different parts of *V. paradoxa*. These parameters were slightly higher in rats treated with bark extracts, followed by those treated with root and leaf extracts. Since, White blood cell are significant in fighting infections and defend the body through phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response, hence rats that received bark extracts of *V. paradoxa* are were able to secrete more antibodies in the process of phagocytosis and adapt to environmental conditions including disease prevalent conditions (Soetan *et al.*, 2013), than those treated with root and leaf extracts. White blood cell are significant in fighting infections and defend the body through phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. Thus, lower WBC counts in rats treated with leaf and root extracts is an indication that the rats were exposed to high risk of infection by the tested microorganisms than the rats treated with bark extracts.

Rats that received root extracts are less likely to develop anaemia due to higher level of Packed Cell Volume (PCV) and haemoglobin (HGB),

report states that Packed Cell Volume and haemoglobin are significant in the diagnosis of anaemia (Chineke *et al.*, 2006).

Conclusion

The present study revealed that leaf, root and bark extracts of *V. paradoxa* showed antidiarrhoea effects against all the tested diarrhoeagenic bacteria. The study also revealed that the bark extracts conferred more protection from diarrhoea more than the leaf and the root extracts of *Vitellaria paradoxa*.

Authors' contributions

FMB and MOB designed the research, FMB performed the research and wrote the paper. MOB and AMO assisted in the verification of the design and reviewed manuscript. All of the authors made substantial contribution to the success of this work and have been involved in revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Acknowledgements

The authors thank the Biological Sciences Department, Federal University of Agriculture Abeokuta.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethic approval and consent to participate

This work was approved by the department of Microbiology Federal University of Agriculture Abeokuta (FUNAAB), Nigeria.

Funding

This study not funded by any individual or bodies, it was self-funded.

Acknowledgement

The authors would like to thank Mr Bankole S.O. for his support in executing this research work.

References

1. Abubakar K, Abdulkadir R, Famoriyo PO (2013). "Evaluation of the antidiarrhoeal effect of

- Vitellaria paradoxa* Gaertn F (Sapotaceae) stem bark extract," *Advances in Life Science and Technology*, 2013. vol. 15, pp. 2224-7181.
2. Akinpelu AO, Amanigbo LEF, Olojede AO (2011). "Health implications of cassava production and consumption," *J Agric. Soc Res.* vol. 11, no. 1, pp 118– 125.
 3. Asuzu IU, Onu OU (1994). "Anthelmintic activity of the ethanolic extract of *Piliostigma thonningii* bark in *Ascaridia galli* infected chickens," *Fitoterapia*, vol. LXV, pp. 291–297.
 4. Awolaye F (1995). "Effects of seed sources on the growth of seedlings of *Vitellaria paradoxa* in the Southern Guinea Savanna of Nigeria," *Nig J. Bot.* vol. 8, pp. 65-69.
 5. Bibitha B, Jisha VK, Salitha CV, Mohan S, Valsa AK (2002). "Antibacterial activity of different plant extracts: A short communication," *Indian Journal of Microbiology* vol. 42, pp. 361-363.
 6. Cheesbrough M (2002). "Medical laboratories manual for tropical countries," Cambridge University Press. pp. 479.
 7. Chineke CA, Ologun AG, Ikeobi CON (2006) "Haematological parameters in rabbit breeds and crosses in humid tropics" *Pakistan Journal of Biological Sciences* 9 (11), 2102 – 2106.
 8. Collinson C, Zewdie-Bosuener A (1999). "Shea butter Markets: Their Implications for Ghanaian Shea butter Processors and Exporters" *A Report 2403*. Natural Resources Institute, University of Greenwich. pp. 20.
 9. Davicino R, Mattar MA, Casali YA, Graciela S, Margarita E, Micalizzi B (2007). "Antifungal activity of plant extracts used in folk medicine in Argentina," *Revista Peruana de Biología*. vol. 14, pp. 247-251.
 10. Duke JA (1992). *Handbook of biologically active phytochemicals and their activities*. CRC Press, Boca Raton, FL. pp. 22-25.
 11. Ebanu RU, Madunagu BE, Ekpe ED, Oung IN (1999). "Microbiological Exploitation of cardiac glycosides and alkaloids from *Garcinia kola*, *Borreria ocymoides*, *kola nitida* and *Citrus aurantifolios*," *J Appli Bacteriol.* vol. 2, pp. 398-401, 1999.
 12. El- Mahmood AM, Doughari JH, LadanN (2013). "Antimicrobial screening of stem bark extracts of *Vitellaria paradoxa* against some enteric pathogenic microorganisms," *Afr. J. Pharm.* Vol. 2, pp. 89-94.
 13. Govindaraji SV, Kannan NK, Jayakumararaj R (2008). "In Vitro Evaluation of Antibacterial Activity Using Crude Extracts of *Catharanthus roseus* L. (G.) Don," *Ethnobotanical Leaflets*. vol. 12, pp. 1013- 1018.
 14. ICRAF (2007). "International Centre for Research in Agroforestry," *Agroforestry Database*. 2000. pp15.
 15. Karou SD, Nadembeg WM, Ilboudo DP. "Medicinal plant with numerous potencies," *Afr. J. Biotech.* vol. 6, pp. 2953-2959.
 16. Kelly BA, Hardy O, Bouvet JM (2004). "Temporal and spatial genetic dynamics in *Vitellaria paradoxa* (shea tree) in an agroforestry system in Southern Mali," *Mol. Ecol*, vol. 13, pp. 1231-1240.
 17. Kumar S, Jenna PK, Sabnam S, Kumari M, Tripathy PK (2010). Antibacteria activity of the flowers of *Woodfordia fruticosa* on different microorganism. *Int. J. Pharm. Sci. Res.* 4: 3225-3228.
 18. Lamien N, Tigabu M, Guinko S, Oden PC (2007). Variations in dendrometric and fruiting characters of *Vitellaria paradoxa* population and multivariate models for estimation of fruit yield. *Agroforestry Syst.* 69: 1-11.
 19. Michiels JA, Kevers C, Pincemail J, Defraigne JO, Dommès J (2012). Extraction conditions can greatly influence antioxidant capacity assays in plant food matrices. *Food Chemistry*. 130 (4): 986-993.
 20. Ndukwe IG, Amupitan JO, Isah Y, Adegke KS (2001). "Phytochemical and antimicrobial screening of the crude extracts from the root, stem bark and leaves of *Vitellaria paradoxa* (GAERTN. F)," *Afr. J. Biotechnol.* Vol. 6, pp. 1905-1909.
 21. Naderi MM, Sarvari A, Milanifar A (2012). "Regulations and ethical considerations in animal experiments: international laws and Islamic perspectives," *Avicenna Journal of Medical Biotechnology*. vol. 4, no. 3, pp.114–120.
 22. Otimenyin OS, Uguru OM, Akanbi BE (2008). "Antidiarrhea Effect of Aqueous Extracts of *Momordica balsamina* and *Stachytarpheta indica* in Rats," *Journal of Natural Products*. vol. 1, pp. 36-45.
 23. Okwu DE, Josiah C (2006). "Evaluation of the chemical composition of two Nigerian Medicinal plants," *African Journal of Biotechnology*. vol. 5, pp. 357-361.
 24. Popoola L, Tee NT (2001). "Potentials of *Vitellaria Paradoxa* Gaertn F. in Agroforestry systems in Benue State," *Nigerian Journal of Ecology*. vol. 16, pp. 20-24.
 25. Saad M, Al-Said A, Al-Barak S (2011). "Extraction of insulin like compound from bitter melon plants," *Am. J. Drugs Disc. Dev.* vol. 1, pp. 1-7.
 26. Saha BK, Sarkar A, Basak R, Chatterjee M (2001). α 25-dihydroxycholecalciferol (Vitamin D3) suppresses the effect of

streptozotocin- induced diabetes during chemical rat liver carcinogenesis. Cell Biol. Int. 25: 227-237.

27. Soetan KO, Akinrinde AS, Ajobade TO (2013). "Preliminary studies on the haematological parameters of cockerels fed raw and processed guinea corn (*Sorghum bicolor*)" in 38th Annual Conference of Nigerian Society for Animal Production, 49-52.
28. Yusha'u M, Bukar A, Balarabe AI (2008). "Prevalence and sensitivity of enterobacterial isolates from patients with urinary tract infections to *Acalypha wilkisenia* extracts," Biological and Environmental Sciences Journal for the Tropics, vol. 5, pp. 72-76.



For Proof Only