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Comparative Phytochemical Investigation of the Various Parts of Vitellaria Paradoxa

M. B. Falana[1*] M. O. Bankole[1] D. A. Ojo[1] A. M. Omemu[2]
1. Department of Microbiology, Federal University of Agriculture, Abeokuta (FUNAAB), P. M. B 2240, Nigeria
2. Department of Food Science and Tourism, Federal University of Agriculture, Abeokuta (FUNAAB), P. M. B 2240, Nigeria

Abstract
Percentage yield of leaf, bark and root of *Vitellaria paradoxa* was determined after extraction with different solvents (methanol, omidun, sterile omidun and water). Quantity of phytochemical constituents in each solvent extract was also determined. The yield range was found highest in methanol extract, followed by omidun extract, sterile omidun and water extracts also in bark extracts (9.33 to 28.80%) followed by root extracts (18.13 to 20.80%) and lowest in leaf extracts (12.00 to 15.46%). Phytochemical results revealed the presence of ten phytochemical constituents at varying quantities but with no significant difference ($p< 0.05$) among all the solvent extracts. Methanol extracts yielded slightly higher amount of phytochemicals followed by sterile omidun, omidun and sterile distilled water extracts respectively. Oxalate was found present in large amount; other classes were present in moderate quantities: tannin, saponin, flavonoid and anthraquinine. While alkaloid, steroid, terpene, phlobatannin and cardiac glycoside were present in small quantities. Though, methanol is a better solvent for extraction, the use of omidun as extraction solvent was neither a bad choice of solvent.
Key words: *Vitellaria paradoxa*, percentage yield, sterile, yield.

1. INTRODUCTION

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al.,* 1999). Medicinal herbs have curative properties and have been used for years in daily life to treat diseases all over the world (Ates and Erdogrul, 2003). The use of herbs and spices in cuisine has been shown as a response to the threat of food-borne pathogens (Cobiac, 2006).

The active components could be normally extracted from all plant structures, but the concentrations of these components vary from structure to structure. The basic parameters influencing the quality of an extract are (Ncube *et la*., 2008) plant part used as starting material, solvent used for extraction and extraction procedure. However, end products of extraction contain complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans (Handa *et al*., 2008).

According to World Health Organization (WHO, 2002), medicinal plants would be the best source to obtain a variety of drugs, this may be 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).Plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Owolabi *et al.,* 2007). Therefore, plants should be investigated to better understand their properties, safety and efficacy (Nascimento *et al*., 2000).

In this study, different solvent extracts of leaf, root and bark of Vitellaria paradoxa (C.F. Gaertn) an indigenous fruit tree belonging to the family Sapotaceae, known locally as Shea butter tree were comparatively assessed for their phytochemical constituents.

2. Materials and Methods

2.1 Collection 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.

2.2. Preparation of Plant Materials

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 (Asuzu and Onu, 1994). The percolates were then filtered and the resulting volume on filtration was reduced to dryness with a Rotary evaporator (RE 100 – Pro) at 45 ± 10ºC. The extracts were then collected, weighed, packed in sterile air tight containers and labeled. They were kept in the refrigerator at 4ºC until needed for analysis.

2.3. Phytochemical analysis

The phytochemicals which are present in the methanol, omidun, sterile-omidun and water extracts of leaf, bark and root of V. paradoxa were determined and quantified by standard procedures as described below (Harborne, 1973; Hagerman *et al*., 2000; Obadoni and Ochuko, 2001; Kumaran and Karunakaran, 2006; AOAC, 2010):

2.3.1. Determination of Total Phenolic Compounds

The total phenolics content in different solvent extracts was determined with the Folin- Ciocalteu's reagent (FCR). In the procedure, 100 mg of extract of the sample was weighed accurately and dissolved in 100 mL of triple distilled water (TDW). One ml of this solution was transferred to a test tube, then 0.5 mL 2N of the Folin-Ciocalteu reagent and 1.5 mL 20% of Na2CO3 solution was added and ultimately the volume was made up to 8 mL with TDW followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid.

2.3.2. Determination of Total Alkaloids

Five grams (5g) of the sample was weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

2.3.3. Determination of total tannins

Five hundred grams of the sample was weighed into a 50 mL plastic bottle. Fifty millimeter of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filterate was pipetted out into a test tube and mixed with 2mL of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

2.3.4. Determination of Total Saponins

The samples were ground and 20 g of each were put into a conical flask and 100 cm3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 mL separatory funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. Sixty millimeter of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated.

2.3.5. Total Flavanoid Determination

Total flavonoid content was determined by Aluminium chloride method using catechin as a standard. One millimeter of test sample and 4 mL of water were added to a volumetric flask (10 mL volume). Five minutes after adding 0.3 mL of 5 % Sodium nitrite, 0.3 mL of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2mL of 1M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 mL with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically (Shimadzu UV-1609, Japan). Results were expressed as catechin equivalents.

2.3.6. Test for Cardiac Glycosides

Five millimeter (5mL) of each extract was treated with 2mL of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underlayed with 1mL concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

2.3.7. Test for Phlobatannins

Deposition of a red precipitate when 2mls of extract was boiled with 1mL of 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

2.3.8. Test for Terpenoids

1ml of chloroform was added to 2mL of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

2.3.9. Test for Quinones
A small amount of extract was treated with concentrated HCL and observed for the formation of yellow precipitate (or colouration).

2.3.10. Test for Oxalate
To 3mL portion of extracts were added a few drops of ethanoic acid glacial. A greenish black colouration indicates presence of oxalates.

2.3.11. Test for Steroids
Steroids were sought by the reaction of Liebermann. Ten mL of ethanolic extract was evaporated. The residue was dissolved in 0.5 mL of hot acetic anhydride; we added 0.5 mL of the filtrate chloroforme. Treated with the reagent of Libermann Burchardt, the appearance, at the interphase, a ring of blue-green, showed a positive reaction

3. RESULTS
3.1. Extraction yields
The extraction with methanol gave the highest extraction yield, for leaf, root and bark of *V. paradoxa* followed by omidun then sterile omidun and lowest percentage yield with water as solvent (Table 1). The yield range was also found highest in bark extracts (9.33 to 28.80%) followed by that of root extracts (18.13 to 20.80%) and lowest in leaf extracts (12.00 to 15.46%).

Table 1. Percentage Yield after Extraction from parts of *V. paradoxa* with different extraction solvents

Plant part	Extraction solvent	Dry Raw powder of Plant part (g)	Plant Extract yield from dry raw powder(g)	Plant Extract Percentage yield%
Leaf	Methanol	150	23.2	15.46
	Omidun	150	21.6	14.40
	Sterile *omidun*	150	19.2	12.80
	Water	150	18.0	12.00
Root	Methanol	150	31.2	20.80
	Omidun	150	28.8	19.20
	Sterile *omidun*	150	24.8	16.53
	Water	150	27.2	18.13
Bark	Methanol	150	43.2	28.80
	Omidun	150	38.4	25.60
	Sterile *omidun*	150	16.0	10.67
	Water	150	14.0	9.33

3.2. Phytochemical assay
Qualitative phytochemical analysis of the extracts revealed the presence of all the tested active ingredients (tannin, saponin, flavonoids, alkaloids, oxalate, steroid, terpene, philoban, cardiac glycoside and anthraquinine) in methanol, omidun, sterile omidun and sterile distilled water extracts of leaf, bark and leaf of *V. paradoxa* (Table 2). The active ingredients were found present in varying proportion (Figures 1, 2 and 3), although there was no significant difference ($p<0.05$) in the quantity of these parameters in all of extracts. Comparatively in order of extracting solvent, the active ingredients were found slightly higher in methanol extract, followed by sterile omidun, omidun and sterile distilled water respectively. Oxalate was found present in large amount and other classes were present in moderate quantities: tannin, saponin, flavonoid and anthraquinine. While alkaloid, steroid, terpene, phlobatannin and cardiac glycoside were present in small quantities.

Table 2: Preliminary phytochemical constituents of methanol, omidun, sterile omidun and water extracts of leaf, bark and root of *V. paradoxa*

Phytochemical Constituents	Methanol	Omidun	Sterile omidun	Water
Tannin	+ve	+ve	+ve	+ve
Saponin	+ve	+ve	+ve	+ve
Alkaloid	+ve	+ve	+ve	+ve
Oxalate	+ve	+ve	+ve	+ve
Flavonoid	+ve	+ve	-ve	-ve
Steroid	+ve	+ve	+ve	+ve
Terpene	+ve	+ve	+ve	+ve
Phlobatannin	+ve	+ve	+ve	+ve
Cardiac glycoside	+ve	+ve	+ve	+ve
Anthraquinone	+ve	+ve	+ve	+ve

+ve Present

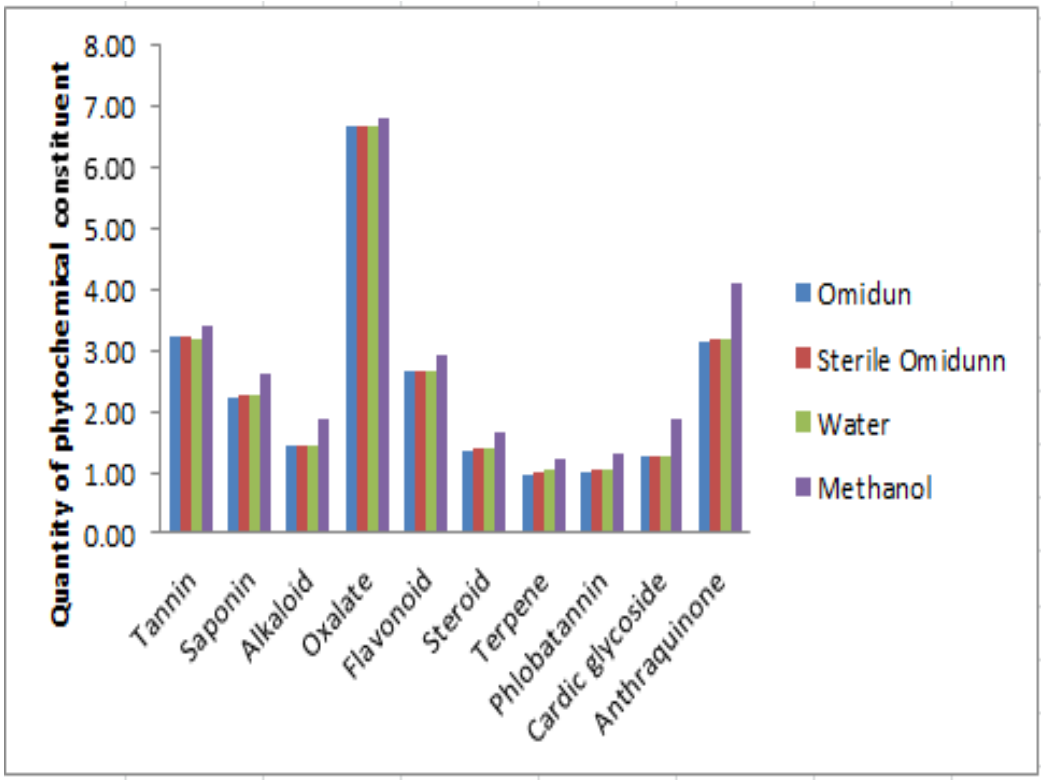

FIGURE 1: Comparative Phytochemical result of leaf extracts of *V. paradoxa* using four solvents: Omidun, Sterile Omidun, Water and Methanol

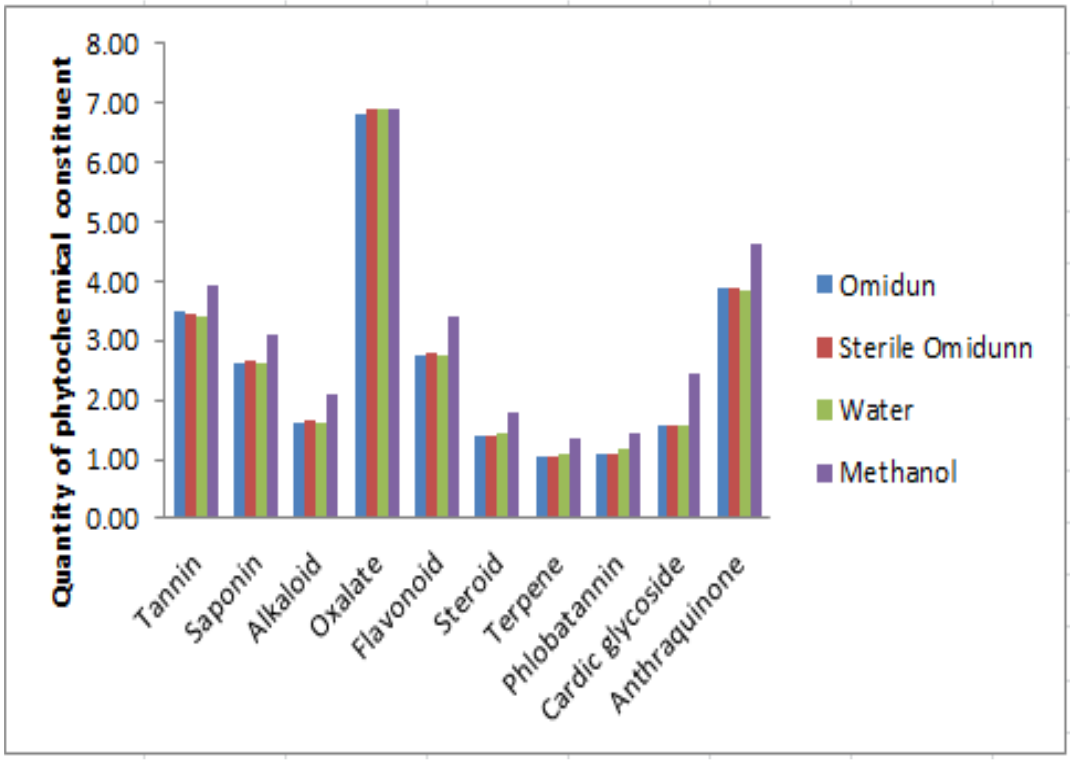

FIGURE 2: Comparative Phytochemical result of bark extracts of *V. paradoxa* using four solvents: Omidun, Sterile Omidun, Water and Methanol

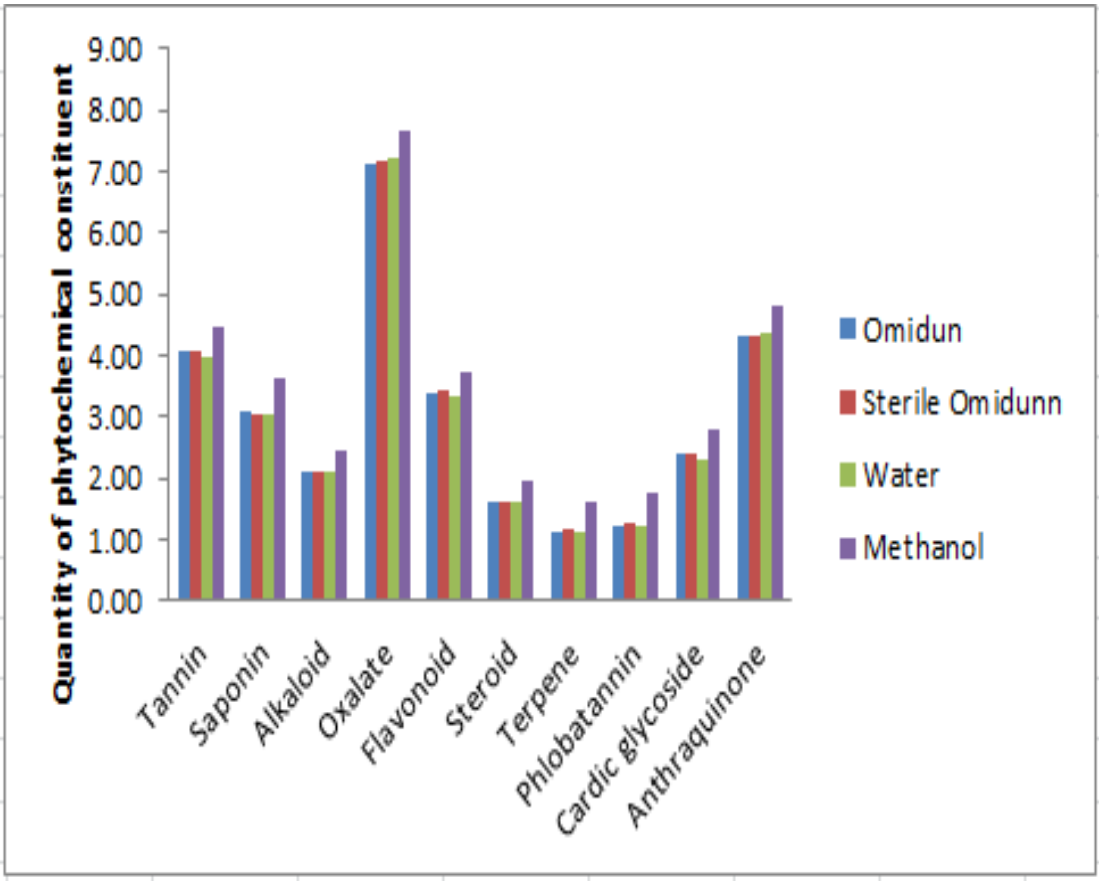

FIGURE 3: Comparative Phytochemical result of root extracts of *V. paradoxa* using four solvents: Omidun, Sterile Omidun, Water and Methanol

4. DISCUSSION

The present study was designed to evaluate the effects of conventional and ethnobotanical techniques of extraction on leaf, root and bark of *V. paradoxa*. Methanol as solvent for extraction of leaf, root and bark of *V. paradoxa* gave highest percentage yield, followed by omidun as solvent then sterile-omidun and lowest percentage yield was with water as solvent (Table). The yield range was also found highest in bark extracts (9.33 to 28.80%) followed by root extracts (18.13 to 20.80%) and lowest in leaf extracts (12.00 to 15.46%). This result is similar to the report by Sun and Ho (2005), where methanol solvent was most effective in extracting active components from oat bran contrary to 52% yield of water extracts of *Senna obtusifolia* obtained by Doughari *et al*. (2007). Owolabi *et al*., (2007) also reported a yield of 10.74% for water extract and 52% methanolic extract of some plants, when compared with the 28.80% methanolic extract of this plant was relatively small and 18.13% water extracts of this plant was very high. Factors like age of the plant and the polarity of the solvent used may have affected the yield. Reports by Ncube *et al.* (2008) and De Boer *et al*., (2005) state that choice of solvent used in extraction of plants may have effect on the yield after extraction and active components of plants are more soluble in organic solvent.

This study has also revealed the presence of ten phytochemical constituents in root, leaves and bark of *V. paradoxa* (Table 2). The constituents are tannin, saponin, flavonoids, alkaloids, steroid, terpene, philoban, cardiac glycoside, phlobatannin and anthraquinone. Onwuliri (2004) have also observed the presence of such constituents as alkaloids, saponins, f1avonoids, tannins, glycosides among others in tropical plants growing in Nigeria. Some of which have been shown to exhibit varying biological activities. They were known to show medicinal activity as well as exhibiting physiological activity (Sofowora, 1993).

However, the phytochemicals were found present at slightly varying proportions (Figures 1 to 3) among solvent extracts of the plant parts. Sofowora (1982) and Onwuliri (2004) have also observed the presence of alkaloids, saponins, flavonoids, tannins, glycosides among others in tropical plants growing in Nigeria. Some of these phytochemicals have been shown to exhibit varying biological activities. Anthraquinone, tannin, saponin and flavonoid were found present in moderate amount while alkaloid, cardiac glycosides, steroid, phlobatannin and terpenes were found very low in the extracts.

Ndukwe *et al.* (2007) evaluated methanol root, stem bark and leaf of *V. paradoxa* for preliminary phytochemicals screening. Tannin, saponin, steroids, alkaloids were found present in all the parts while phlobatannin, cardiac glycosides and flavonoids were found absent in the plant parts which were found present in small quantities in the present study (Figures 1, 2 and 3). This agrees with the phytochemical findings of Konkon *et al*. (2008) who found flavonoid, tannins and alkaloids in methanol solution of leaf extract of *V. paradoxa*. However, saponins were found to be absent in his report but found present in this study. The presence of these phytochemical constituents may be responsible for some of the observed antibacterial activity observed in this study.

Mensah (2008) reported the importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains. Also, phytochemicals, such has alkaloids, tannins, flavonoids and saponin have been known to produce a definite physiological action on human body (Edeoga, 2005). The presence of cardiac glycosides indicates that they may act as good sedatives and have antispasmodic properties (Egunyomi, 2009). Hence, the presence of phytochemical constituents such as saponins, tannins, alkaloids and steroids in the plant parts under study is an indication that the plant is of potential antimicrobial agent, hence it of pharmacological importance (Hostettmann, 1995).

5. Conclusion

From this study, it can be concluded that methanol, omidun, sterile omidun and water extracts of leaf, bark and root of *V. paradoxa* contain phytochemicals that have been reported to possess antimicrobial properties. Hence, this study support the fact that parts of *V. paradoxa* can be seen as potential sources of useful drugs. However, more work needs to be done on the purification, identification and quantification of active components of leaf, root and bark of *V. paradoxa* with the view to develop new antimicrobial agents that address the antimicrobial resistance problems.

Conflict of Interests

The authors declare that there is no conflict of interests.

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References

Hammer, K.A., Carson, C.F. and Riley, T.V. (1999). Antimicrobial activity of essential oils and other plant

extracts *J. Appl. Microbiol*. 86: 985-990.
Ates, D. A. and Erdogrul, O. T. (2003). Antimicrobial activities of various medicinal and commercial plant extracts. *Turk. J. Biol,* vol. 27, pp. 157-162, 2003.
Cobiac, L., Patch, C.S., Sullivan, D.R., Fenech, M, Roodenrys, S., Keogh, J.B., Clifton, P.M., Williams, P.G., Fazio, V.A., Inge, K.E. and Tapsell, L.C. (2006). "Health benefits of herbs and spices: the past, the present, the future". 185: 4-24pp.
Ncube, N., Afolayan, S.A. and Okoh, A. I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *Afr. J. Biotechnol*. 7: 1797-1806.
Handa, S.S., Khanuja, S.P., Longo, G, and Rakesh, D.D. (2008). Extraction technologies for medicinal and aromatic plants. Trieste. 21-25pp
WHO (2002). Traditional Medicine: Growing Needs and Potential. WHO Policy Perspectives on Medicines. World Health Organization, Geneva pp. 1-6.
Kumar, S. Jenna, P.K., Sabnam, S., Kumari, M. and Tripathy, P.K. (2010). Antibacteria activity of the flowers of Woodfordia fruticosa on different microorganism. *Int. J. Pharm. Sci. Res*. 4: 3225- 3228.
Owolabi O.J., Omogbai E.K.I. and Obasuyi O. (2007). Antifungal and antibacterial activities of the ethanolic and aqueous extract of Kigella africana (Bignoniaceae) stem bark. *Afr. J. Biotechnol*. 6: 882-885
Nascimento, G. F., Locatelli, J., Paulo, C.F. and Silva, G. (2000). The antimicrobial activity of plant extract and phytochemical on antibiotic resistant bacteria. *Brazil J. Microbiol*. 31: 247-256.
Asuzu, I.U. and Onu, O.U. (1994). Anthelmintic activity of the ethanolic extract of Piliostigma thonningii bark in Ascaridia galli infected chickens. *Fitoterapia.* LXV: 291–297.
Harborne, J.B. (1973). Phytochemical methods. Chapman and Hall. London. 1–32pp
Hagerman, A., Harvey-Mueller, I. and Makker, H.P. (2000). Quantification of tannins in the foliage-a laboratory manual. FAO/IAEA,Vienna, Austria. 4-7pp.
Obadoni, B.O. and Ochuko, P.O. (2001). Phytochemical studies andcomparative efficacy of the crude extract of some homeostatic plantsin Edo and Delta states of Nigeria. Global *J.Pure Appl. Sci*. 8: 203-208.
Kumaran, A. and Karunakaran, J. (2006). *In vitro* antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT-*Food Sci. Technol*. 40: 344-352.
AOAC, (2010). Official Methods of Analysis. Association of Official Analytical Chemists, *15th Edn. Washington D.C., USA*. 184pp
Sun T, Ho C (2005). Antioxidant activities of buckwheat extracts. *Food Chem*., 90: 743–749.
Doughari, J. H., El-mahmood, A. M. and Manzara, S. (2007). Studies on the antibacterial activity of root extracts of *Carica papaya L. Afri. J. Microbiol. Res*. 037-041.
De Boer, H. J., Kool, A. and Broberg, A. (2005). Antifungal and antibacterial activity of some herbal remedies from Tanzania. *J. Ethnopharmacol*. 96: 461-469.
Onwuliri, F. C. (2004). Antimicrobial studies of the extracts of *Acalypha wilkesiana L*. on microorganisms associated with wound and skin infections. *West African Journal of Biological Science*, vol.15, pp. 15-19.
Sofowora, A. (1993). Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, pp. 191-289.
Sofowora, A. (1982). Medicinal plants and traditional medicine. John Wiley and Sons Ltd. New York.
Ndukwe, I. G., Amupitan, J.O., Isah, Y. and Adegoke, K.S. (2007). Phytochemical and antimicrobial screening of the crude extracts from the root, stem bark and leaves of Vitellaria paradoxa (GAERTN. F). *Afr. J. Biotechnol*. 6: 1905-1909
Konkon, N. G., Adjoungouna, A. L. Manda, P. Simaga, D. N'Guessan, K. E. and Kone, B. D. (2008). Toxicological and phytochemical screening of Mitragyna inermis (Wild) O Ktze (Rubiaceae), antidiabetic plant. *J. Med. Plant. Res.* 2: 279-284.
Mensah, J. K., Okoli, R. I., Ohaju-Obodo, J. O. and Eifediyi, K. (2008). Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. *African Journal of Biotechnology*. 7: 2304-2309.
Edeoga, H. O. Okwu, D. E. and Mbaebie, B. O. (2005). "Phytochemical constituents of some Nigerian medicinal plants", *Afr. J. Biotechnol*, Vol. 4, pp. 685-688.
Egunyomi, A., Moody, J.O. and Eletu, O.M. (2009). Anti-sickling activities of two ethnomedicinal plant recipes used for the management of sickle cell anaemia in Ibadan Nigeria. *Afr. J. Biotechnol*. 8: 20-25.
Hostettmann, K. (1995). Strategy for the biological and chemical evaluation of plant extracts. *Pure Appl. Chem*, vol. 70, pp.1-9.