IN SILICO AND IN VITRO ANTIMICROBIAL TESTING OF AQUEOUS EXTRACT OF Vernonia amygdalina AGAINST Escherichia coli ISOLATED FROM WISTAR RATS INFECTED WITH Trypanosoma congolense

Tom-Otu, M. O., Omowaye, O. S., Makolo, D., Ayodele P. F., Ogunyemi, O. M. & Fabusiwa, M.

Department of Biosciences, College of Natural and Applied Sciences, Salem University, Lokoja, Kogi State, Nigeria.
Department of Microbiology, Faculty of Natural and Applied Sciences, Veritas University, Abuja, Nigeria.
Medical Services Department, Medical Laboratory Division, Ajaokuta Steel Company Limited, Ajaokuta, Kogi State, Nigeria.
Department of Biochemistry, College of Natural and Applied Sciences, Salem University, Lokoja, Kogi State, Nigeria.

Corresponding Author Email: ayodele.peter@pg.funaab.edu.ng

ABSTRACT

The study was carried out to investigate the in silico and in vitro antimicrobial activity of Vernonia amygdalina aqueous extract (VAAE) against Escherichia coli on rats already infected with Trypanosoma congolense. Forty Wistar rats of 16 weeks old were used, and were randomly selected into six groups, where n = 5 in groups A, B, D, E and F; n = 15 in group C. Group A: uninfected-untreated; group B: uninfected-treated (500 mg/kgbwVAAE); group C: infected-untreated; groups D: infected-treated (100 mg/kgbw VAAE), E: infected-treated (300 mg/kgbw (VAAE) and F: infected-treated (500 mg/kgbw (VAAE). Using docking simulation, a number of phytochemicals were screened and identified in the VAAE used in the study. The findings have given an indication of in vitro antimicrobial activity of aqueous extract of Vernonia amygdalina leaves against Escherichia coli. Docking with topoisomerase iv (3FV5) revealed that 3 compounds (−8.5 to −8.3 kcal/mol) with docking scores lower than the co-crystalized PBE (−6.1 kcal/mol). The five topmost docked compounds including vernonioside A22, vernonioside A42, luteolin-7-rutinoside, vernonioside D2 and proline betaine (PBE) with the lowest docking scores exhibited the highest binding tendency with topoisomerase iv, upon interaction analysis. Residues involved in the intermolecular interaction include Asp69, Ile116, Ser117, Leu94, Gly73 Leu89, Arg93, Ile90, Glu46 and Val39 (h-bonds), Val118, Ile90, Met74, Gly73, Arg72, Pro75, Val39, Val165 and Tyr505 (π-π stacking interactions) and Glu46. Our findings revealed the stability of vernonioside A22, vernonioside A42, luteolin-7-rutinoside, vernonioside D2 and proline betaine (PBE) on the target topoisomerase iv in the target organism, which in support of the in vitro study has also shown that Vernonia amygdalina leaves have antimicrobial potential and as such be considered as a formidable source for the search for new drugs against bacteria.

Keywords: Antimicrobial activity of Vernonia amygdalina, Bitter leaves, Escherichia coli, Molecular simulation, Vernonia amygdalina, Vernoniosides

LICENSE: This work by Open Journals Nigeria is licensed and published under the Creative Commons Attribution License 4.0 International License, which permits unrestricted use, distribution, and reproduction in any medium, provided this article is duly cited.
COPYRIGHT: The Author(s) completely retain the copyright of this published article.
OPEN ACCESS: The Author(s) approves that this article remains permanently online in the open access (OA) mode.
QA: This Article is published in line with “COPE (Committee on Publication Ethics) and PIE (Publication Integrity & Ethics)”. 
INTRODUCTION
Over the years, there has been success of complementary and alternative medicines in the treatment of infectious diseases, indicating the effectiveness of plants in the treatment of different types of microbial diseases such as bacterial, parasitic, fungal, and viral diseases (El-Hajj and Holst, 2020). Suffice it to say that in almost every human society, medicinal plants have been a source of medicine for ailments and over 50% of all modern clinical drugs are of natural products origin. Similarly, in Nigeria, traditional plants occupy considerable space in the medical system, and parts of the plants continue to be a major resource to resist serious diseases globally (Ayodele et al., 2021a,b; Ari-egoro et al., 2019; Zubairu, 2019).

*Vernonia amygdalina* has gained wide application in the treatment of amoebic dysentery (Muhammed et al, 2019), gastrointestinal disorders and antiparasitic activities. The works of Udochukwu et al. (2015); Ogundare (2011) indicated that the leaf extracts of bitter leaf possess inhibitory potentials against some selected microorganisms.

Bacterial infections are exerting difficulties on humans and animals worldwide, principally because of the expansion of antibiotic resistance (Zubairu et al., 2019). In recent times, as reported by Gurbani et al. (2016), there has been a rise in the drug resistance challenge of gram-negative bacteria and multi-drug resistant (MDR) organisms, such as *Escherichia coli* (*E.coli*) amongst others. *E.coli* is common and a diverse group of bacteria found in food, the environment and in the intestines of both humans and certain warm-blooded animals (such as the ruminants). *E.coli* are pathogenic, causing diarrhea, urinary tract infection, pneumonia, cholecystitis, or septicemia (Xiao-Nan et al., 2015). However, no report yet has shown the efficacy of the antimicrobial property of *Vernonia amygdalina* against *E. coli* in infected rats.

MATERIALS AND METHODS
Study Area
This study was carried out in the Microbiology and Biochemistry Laboratories, Salem University, Lokoja, Kogi State, Nigeria.

Sample Collection
Fresh leaves of *Vernonia amygdalina* were harvested from a local garden in Ajaokuta Local Government Area of Kogi State, Nigeria. The botanical identification and authentication of the plant (SU-H-0056) were done by a Botanist in the Biological Department of Salem University. The leaves were carefully detached and air-dried until crispy to touch and then pulverized into a fine powder using a sterile electrical blender (Euro premium: 1500-1799 W).

Preparation of Plant Extract
The *Vernonia amygdalina* powder was weighed with a Camry automatic scale with model No: Ek3250 (A & D Company Limited, Japan) into a clean dry flat-bottom flask. Twenty-five grams (25 g) of the pulverized sample was cold macerated in 250 ml distilled water (1: 10 w/v) for 48 hrs with constant rocking using an electronic shaker with model Ng: 001 (Ayodele et al. 2021b). The ethanol extract was filtered using Whatman No. 1 filter paper, a pore size of 100 (195 mm by 195 mm). The filtrate was concentrated using a rotary evaporator and placed on a water bath at
60°C to allow evaporation of the solvents as described by AOAC (2010). The obtained jelly-like extract was stored in a sterile Petri dish in the refrigerator at 4°C until required for use.

**Sterility of Plant Extract**

The sterility of the extract was confirmed following the standard laboratory procedure. *Vernonia amygdalina* leaves extract was inoculated on sterile nutrient agar and incubated at 37°C for 24 hrs. The absence of microbial growth on the extract after the incubation confirmed the sterility of the extract.

**Experimental Animals**

Forty (40) healthy male Wistar rats 16 weeks old were purchased from the Nigerian Institute of Trypanosomiasis Research (NITR), Jos, Plateau State, Nigeria. The animals were acclimatized and kept in the animal house of Salem University, Lokoja, Nigeria, where they were maintained under 12 hrs (light/dark) cycle where they were allowed free access to a pellet diet and clean water *ad libitum*. All procedures used complied with the guide for the care and use of laboratory animals following good laboratory practice (GLP) regulations of the World Health Organization (WHO). The principles of laboratory animal care were also duly followed.

**Experimental Design**

The forty male Wistar rats of 16 weeks were randomly selected into six groups, where n = 5 in groups A, B, D, E and F; n = 15 in group C. Group A: uninfected-untreated; group B: uninfected-treated (500 mg/kgbw VAAE); group C: infected-untreated; groups D: infected-treated (100 mg/kgbw VAAE), E: infected-treated (300 mg/kgbw (VAAE) and F: infected-treated (500 mg/kgbw (VAAE). Infection of the experimental rats was carried out by injecting 0.1 ml of blood containing approximately 1 ×10^3 trypanosomes intraperitoneally into each rat in the infected groups. The experiment lasted for 7 days.

**Culture Media Preparation**

The culture media used in the study include eosin methylene blue (EMB), mannitol salt agar (MSA), nutrient agar (NA), and Mueller Hinton Agar (MHA). All media were prepared according to their manufacturers’ instructions. The media were autoclaved at 121°C for 15 mins as described by Sandle (2017).

**Bacteria Isolates**

Pure cultures of the *E. coli* were obtained from the experimental animals by the laboratory standard methods of culturing and subculturing. A sterile swab stick and wire loop were used to collect samples from the faeces of each test animal. The Presumptive *E. coli* sample (from the faeces) was inoculated using a sterile wire loop, into an EMB agar plate and incubated at 37°C for 24 hrs. The organism was authenticated using the method of Patel (2017). Culture and morphological characterization were carried out on the organisms, on selective media; gram staining technique, and biochemical reactions according to Doaa *et al.* (2012).
Identification and Characterization of Test Organisms

Gram Staining Reaction
The bacteria cell was subjected to a gram staining procedure as documented by Doaa et al. (2012).

Biochemical Tests to Identify E. coli
Biochemical tests such as coagulase, catalase, oxidase, urease, indole, fermentation and citrate utilization tests were done to identify the test organism according to Doaa et al. (2012).

Antibacterial Susceptibility Testing of VAAE
The method of Zubairu et al. (2019); Makolo et al. (2019) was adopted in performing the antibacterial susceptibility tests using the standard disc diffusion technique. Sensitivity discs were made of Whatman No. 1 filter paper (6 mm in diameter). The sterile discs were impregnated with different concentrations of the extract (10 mg/ml to 50 mg/ml) and were placed aseptically on Mueller Hinton agar that has earlier been inoculated with the test organism. A set of Gram-negative and Gram-positive control discs using some agar seeded with the test organisms were also set up. All plates (test and control) were incubated overnight at 37°C for about 24 hrs. The zones of inhibitions were measured according to the method of Jan (2009). Values were recorded in mm.

Preparation of Protein Structures of Topoisomerase iv in E. coli
The crystal protein structure of topoisomerase iv of E. coli was retrieved from the Protein Data Bank (PDB ID: 3FV5). From the retrieved structure, the native ligands were extracted, and water molecules were removed. Hydrogen atoms were added to the structures using Autodock version 4.2 programs (Scripps Research Institute, La Jolla, CA).

Preparation of Ligand Structures of VAAE
The 3D structures of compounds derived from the plant and reference compounds were retrieved from the PubChem database (www.pubchem.ncbi.nlm.nih.gov) in the structure data format (SDF). The SDF structures of these compounds and reference compounds were converted to mol-2 chemical format using OpenBabel. The polar hydrogen charges of the Gasteiger-type were assigned to atoms in the chemical structure, and the non-polar molecules of hydrogen were merged with the carbons. The internal degrees of freedom and torsions were set to zero. The structures were then converted to the dockable PDBQT format utilizing AutoDock tools.

Molecular Docking Simulations of VAAE against Topoisomerase iv of E. coli
The ligand structures were imported into AutoDock Vina in PyRx 0.8 (Trott and Olson, 2010) and minimized using the incorporated OpenBabel by applying the Universal force field (UFF) as the energy minimization parameter and conjugate gradient descent as the optimization algorithm. The ligand structures were then screened against the active site of topoisomerase iv. The active site of the protein was defined by the grid boxes. The molecular docking simulations were then analyzed keeping all other parameters as default. After docking simulation, the molecular interactions between the ligands and proteins were viewed with discovery studio visualizer version 16.
Molecular Dynamics Simulation

The apo enzymes (PDBID), and the lead compounds complexed with the protein were subjected to full atomistic Molecular Dynamic (MD) simulation using GROMACS 2019.2 and GROMOS96 43a1 forcefield on the WebGRO server (Abraham et al., 2015; Oostenbrink et al., 2004). The ligands topology files were generated using the PRODRG webserver (http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg). The enzymes and ligand-enzyme complex systems were solvated within a cubic box of the transferable intermolecular potential with a four-point (TIP4P) water model, applying the periodic boundary conditions at a physiological concentration of 0.154 M set by neutralized salt ions. The minimization of the systems was performed for 10000 steps using the steepest descent algorithm constant number of atoms, volume, and temperature ensemble (NVT) ensemble for 0.3 nanoseconds, followed by 0.3 nanoseconds of equilibration in constant atom number, constant pressure and constant temperature (NPT). The temperature was maintained using 310 K using velocity rescale, while pressure was set to 1 atm using Parrinello-Rahmanbarostat. Leap-frog integrator was used with a time step of 2 femtoseconds. For each system, 100 ns of the production run was performed and for every 0.1 ns, a snapshot was saved with a total of 1000 frames from each system. The trajectories were analyzed using VMD TK console scripts. to calculate RMSD, RMSF, SASA, RoG, and the number of H-bond. Amino acid interactions of selected phytocompounds with Topoisomerase iv of E.coli were also simulated.

Data Analysis

Data were analyzed using graph prism (V: 20). Data were expressed as Mean ± SEM. One-way analysis of variance and significant means were separated by post hoc Duncan’s multiple range tests at p-value < 0.05.

Ethical Approval

The Nigerian Institute of Trypanosomiasis Research (NITR), Jos, Plateau State, Nigeria authorized the fieldwork.

RESULTS

Zones of inhibition produced by VAAE on E. coli

Figure 1 represents the zones of inhibition produced by VAAE on E. coli. The aqueous extract of the VAAE exhibited antibacterial potentials: E. coli (8-20 mm).
Figure 1: *In vitro* antimicrobial effect of VAAE on *E. coli*. All determinations were conducted in triplicates; data were expressed as mean±SEM.

**Binding Affinity of Vernonia Compounds with Topoisomerase IV of *E. coli***

The binding affinities from the docking analysis of the protein topoisomerase IV (3FV5) for the phytocompounds used against *E. coli* are shown in Table 1. Based on the minimum binding energies and interactions with catalytic residues, the top five phytocompounds with binding energies ranging from (−8.5 to −8.1 kcal/mol) were compared with the binding energies of the reference compounds 1-(4-acetyl-6-pyridin-3-yl-1H-benzimidazole-2-yl)-3-ethyl urea(PBE) (−6.1 kcal/mol). From the interaction of the top phytocompounds with topoisomerase iv, three compounds with the lowest binding energies compared to the reference compound were selected: vernonioside A22, vernonioside A42, luteolin-7-rutinoside, two phytocompounds that had higher binding energies to that of the reference compound were selected: vernonioside D2 and vernonioside B32, as shown in Table 1 and 2.

**Table 1:** Binding energy scores for the interactions between selected screened phytocompounds in VAAE and topoisomerase iv of *E. coli*

| S/NO | Phytocompounds       | Binding Energy score (Kcal/mol) 3FV5 |
|------|----------------------|-------------------------------------|
| 1    | Vernonioside A22     | −8.5                                |
| 2    | Proline betaine (PBE)| −8.3                                |
| 3    | Luteolin-7-rutinoside| −8.3                                |
| 4    | Vernonioside A42     | −8.3                                |
| 5    | Vernonioside D2      | −8.2                                |
| 6    | Vernonioside B32     | −8.1                                |
| 7    | Luteolin             | −7.2                                |
| 8    | Neoandrographolide    | −7.2                                |
| 9    | Myricetin            | −7.0                                |
| 10   | Apigenin             | −7.0                                |
| 11   | Pinocarveol-2        | −4.8                                |
| 12   | Trans-pinocarveol    | −4.5                                |
| 13   | Vernolic             | −4.5                                |
| 14   | 2-Methyl-3-hexanol   | −4.1                                |
| 15   | 2,3-Pentanedione     | −3.7                                |
| Compounds          | Enzyme | Hydrogen bonds interactions (Bond distance Å) | Hydrophobic Interaction | Other interactions |
|--------------------|--------|-----------------------------------------------|-------------------------|--------------------|
| Vernioniose A22    |        | ILE116(2.10; SER117(2.48) ASP69(2.53) GLU46(2.15) LEU94(3.72) | 1 VAL118(4.93) | 0 None            |
| Vernioniose A42    |        | GLY73(2.46) ASP69(2.26; GLU46(2.76) GLU82(2.00) | 1 ILE90 (4.50) | 0 None            |
| Luteolin-7-rutinoside | 3FV5 | GLY73(2.39) ASP69(2.86; ILE90(3.49) LEU89(3.74) | 3 ILE90 (4.55; 5.44 | 1 GLU46(4.23) |
| PBE                |        | ASP69(2.44; VAL39(3.78) | 6 MET74(4.00; 4.35; 4.35; 4.35) GLY73(4.35; 4.35) ARG72(4.56) PRO75(4.53; 4.81) VAL39(5.10) VAL165(4.90) | 1 GLU46(4.48; 4.24) |
| Vernioniose D2     |        | ILE116(2.71;2.10) SER117(2.31; 2.58) | 4 ALA86(4.23) ILE90 (5.18) ARG93(4.54) LEU89 (4.01) | 0 None            |
| Vernioniose B32    |        | ARG93(2.58) ILE116(2.60; 2.82) ASN42(2.46) | 6 ALA86(4.21) ILE90 (4.52; 4.30 4.52) PRO75(4.85) HIS79(4.57) | 0 None            |

**Amino Acid Interaction of Selected Phytocompounds with Topoisomerase iv of *E.coli***

The amino acid interactions of topoisomerase iv with reference compound and five ranked phytocompounds that demonstrated the highest binding tendencies are represented in Figs. 12–17 showing the 2D and 3D structures. The interacting residues of the protein with respective ligand groups were majorly through H-bond, hydrophobic interactions, and a few other bonds (Table 2 of the supplementary file). In the Vernioniose-A22-3FV5 complex, a conventional hydrogen bond and carbon-hydrogen bond were formed with GLU46, ASP69, SER117, ILE116 and LEU 94 respectively while the alkyl interaction was formed with VAL118 (Fig. 12). In the Vernioniose-A42-3FV5 complex a conventional hydrogen bond were formed with ASP69, GLY73, GLU46 and GLU82 respectively while the alkyl interaction was formed with ILE90 (Fig. 13).In the luteolin-7-rutinoside-3FV5 complex, a conventional hydrogen bond and carbon hydrogen bonds were formed with ASP69, GLY73, LEU89 and ILE90 respectively while the alkyl interaction was formed with MET74 and other interactions with GLU46 (Fig. 14). In the vernioniose-D2-3FV5 complex, conventional hydrogen bonds were formed with SER117 and ILE116 respectively while the alkyl interaction was formed with ALA86, LEU89, ILE90 and ARG93 (Fig. 15). In the vernioniose-B32-3FV5 complex, a conventional hydrogen bond was formed with ARG93, ASN42 and ILE116 respectively while the alkyl interaction was formed with ALA86, HIS79, ILE90 and PRO75 (Fig. 16). In the PBE-3FV5 complex, a conventional hydrogen...
bond and carbon hydrogen bonds were formed with ASP69 and VAL39 respectively while the alkyl interaction was formed with PRO75, VAL165, ARG72, GLY73 and GLU46.

**Figure 2:** The structure of the vernonioside-A22-3FV5 complex. 3D(a) 2D(b)

**Figure 3:** The structure of the vernonioside-A42-3FV5 complex. 3D(a) 2D(b)
Figure 4: The structure of the luteolin-7-rutinoside-3FV5 complex. 3D(a) 2D(b)

Figure 5: The structure of the vernonioside-D2-3FV5 complex. 3D(a) 2D(b)
Figure 6: The structure of the vernonioside-B32-3FV5 complex. 3D(a) 2D(b)

Figure 7: The structure of the PBE-3FV5 complex. 3D(a) 2D(b)
Figure 8: The root mean square deviation of topoisomerase IV in complex with phytochemicals from Vernonia amygdalina

SASA

RMSD
**Figure 9:** The surface accessible surface area of topoisomerase iv in complex with phytochemicals from *Vernonia amygdalina*

**Figure 10:** The root mean square fluctuation of topoisomerase iv in complex with phytochemicals from *Vernonia amygdalina*
DISCUSSION

Susceptibility test is to evaluate the resistance or sensitivity of pathogenic aerobic and facultative anaerobic bacteria to several antimicrobial phytochemicals or compounds, to assist in the selection of drug therapeutic options (Jan, 2009). In this study, *E. coli* was observed to be sensitive to all the concentrations of the VAAE. This study supported the reports of Udochukwu *et al.* (2015); Ogundare (2011) who reported that the extracts of *Vernonia amygdalina* (bitter leaves) exerted inhibitory effects on *E. coli* and *Staphylococcus aureus*. However, our findings are not in agreement with the report of Evbuomwan *et al.* (2018) who reported that *E. coli* was observed to be completely resistant irrespective of the bitter leaf extract concentrations. The VAAE was not inhibitory to the test organisms, even at its lower concentrations.

Chea *et al.* (2021) have attributed the difference in the antibacterial effect between Gram-positive and Gram-negative organisms to the structural composition of the bacterial cell. Gram-positive bacteria (*S. aureus*) have a relatively thick peptidoglycan layer that is fully permeable to substances, thus making it more sensitive to the extract (Evbuomwan *et al.*, 2018). Gram-negative bacteria have thick lipopolysaccharide bilayers embedded with carrier proteins whose channel size determines the size of molecules to pass through. The larger size of the plant bioactive compounds may not be able to pass through into the bacterial cell, thus being less sensitive to extracts.

Parallel advances in protein crystallography and various virtual screening software for the modeling of ligand-receptor interactions have enhanced computer-aided drug design. In this study, a structure-based virtual screening of
phytocompounds was employed via a competitive docking approach for topoisomerase iv agonist with a dual inhibitory potential against *E. coli*. The top five phytocompounds for topoisomerase iv were further analyzed for antibacterial effect and they were competitively and selectively docked. They were docked into the hydrophobic ligand binding pocket (LBP) which is located in the bottom half of the GR ligand binding domain, LBD (Morris *et al.*, 2009; Trott and Olson 2010). The top five compounds were vernonioside A22, vernonioside A42, luteolin-7-rutinoside, vernonioside D2 and vernonioside B32(-8.5, -8.3-8.3, -8.2, -8.1kcal/mol) and the reference compound (PBE) had -8.3kcal/mol. These phytocompounds present might be responsible for the pharmacological property of *Vernonia amygdalina*, exerting the antimicrobial effect, as it is relative to the report of Udochukwu *et al.* (2015); Ogundare (2011).

**CONCLUSION**

This study has indicated the *in vitro* antimicrobial activity of aqueous extract of *Vernonia amygdalina* against *Escherichia coli*. The results acquired from this study showed that vernonioside phytocompounds (vernonioside A22, vernonioside A42, luteolin-7-rutinoside, vernonioside D2 and proline betaine) obtained from *Vernonia amygdalina* showed effective interaction with the topoisomerase iv of *E. coli*, making the plant promising enough to exert an antimicrobial effect. This study thus has shown that *Vernonia amygdalina* (bitter leaf) could have antimicrobial potentials and as such be considered a formidable source for the search for new drugs against bacteria, hence the need for further research on better methods of extraction such as activity-guided fractionation of the crude extract to establish and concentrate the fraction where the bioactivity against bacteria lies and the degree of the antimicrobial potential of *Vernonia amygdalina*.

**CONFLICT OF INTEREST**

The authors declare no conflicts of interest

**ACKNOWLEDGEMENTS**

The Department of Microbiology, College of Natural and Applied Sciences, Salem University, Lokoja Nigeria, is acknowledged for providing the basic equipment and infrastructure for carrying out the research work.

**AUTHORS’ CONTRIBUTION**

**Mary Otuhuo Tom-Otu**: Methodology, Investigation, Resources Project administration, Writing- original draft.

**Olaniyi Stephen Omowaye**: Conceptualization, Methodology, Analysis, Investigation, Resources, Supervision, Project administration, Writing- review and editing.

**Daniel Makolo**: Methodology, Investigation, Resources, Project administration, Writing- review and editing.

**Peter Folorunsho Ayodele**: Methodology, Investigation, Resources Project administration, Writing- review and editing.
REFERENCES
Abraham, M. J., Murtola, T., Schulz, R. (2015). GROMACS: High performance molecular simulations through multilevel parallelism from laptops to supercomputers. SoftwareX, 1-2: 19-25.

Ari-egoro, Y. E., Ayodele, P. F. and Oyeleke, O. M. (2019). Effects of graded levels of Moringa oleifera leaf-meal in albino rat diet on some hematological parameters. Journal of Analytical Technique and Research, 1(2): 37-46.

Association of Official Analytical Chemists. (2010). Official methods of analysis of AOAC international, 18th edition, Washington DC.

Ayodele, P.F., Dorcas Ibukun Akinloye, Jamiu Akamo Adio, David Adejare Agboola, Oluseyi Adeboye Akinloye (2021). Effect of ethanolic extract of Xylopia aethiopica fruit on cadmium-induced inflammatory changes and dyslipidemic rats. Asian Journal of Research in Biochemistry, 9(4): 9-16a.

Ayodele, P.F., Oyeleke, O., Onifade, O.F., Ifeoluwa,O.A., Omowaye, O.S. and Muhammad, A. (2021). Nutritional evaluation of Moringa oleifera leaves and the effect of its biofortification with animal feed on physical changes and organ weight in male albino rats. Asian Journal of Research in Biochemistry, 9 (4): 1-8b.

Chea, S. K. P., Veselee, K., Mulbah, E., Borlay, A. J. and Javam M. M. (2019). Antibacterial potential of Vernonia amygdalina plant against Staphylococcus aureus and Escherechia coli. Asian Journal of Science and Technology. 10(11): 10431-10434.

Doaa, E. and Saliwa, A. (2012). Identification of Staphylococcus aureus and Escherechia coli isolated from Egyptian food by conventional and molecular methods. Journal of Genetic Engineering and Biotechnology, 10(1): 129-135.

El-Hajj, M. and Holst, L. (2020). Herbal medicine use during pregnancy: a review of the literature with a special focus on sub-Saharan Africa, In Frontiers in Pharmacology, 11: 66-88.

Evbuomwan, L., Chukuka, E. P., Obazenu, E. I., and Ilevbare, L. (2018). Antibacterial activity of Vernonia amygdalina leaf extracts against multidrugs resistant bacteria isolates. Applied Sciences and Environmental Management, 22(1): 17-21.

Gurbani, K., Rahul, K., Shruti, B. an Archana, S. (2016). A review on dietary antioxidants and their indispensable role in periodontal health. Journal of Food and Drug analysis, 1: 1-8.

Jan, H. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. American Society for Microbiology, 15:55-63.
Makolo, D., Suleiman, A. B., Olonitola, O. S., Bello M., Alfa, M. I., Ahmadu, I. and Awulu, F. O. (2019). Antimicrobial susceptibility profile of coliforms from Bovine mastitis cases among pastoral Herds in parts of Kaduna State, Nigeria: Curbing the environmental health risk. *Asian Journal of Advanced Research and Reports, 3*(2): 1-12.

Muhammed, A., Sani, U., Diso, S. A W. and Muhammad, S. A. (2019). Phytochemical screening and antibacterial activity of bitter leaf (*Vernonia amygdalina*). *Annals of Microbiology and Infectious Diseases, 2*(4): 2637-5346.

Ogundare, A. O. (2011). Antibacterial properties of the leaf extracts of *Vernonia amygdalina, Ocimum gratissimum, Corchorus olitorius* and *Manihot palmate*. *Journal of Microbiology and Antimicrobials, 3*(4): 77-86.

Oostentbrink, C., Villa, A., Mark, A. E. (2004). A biomolecular forced field based on the free enthalpy of hydration and salvation: the GROMOS force-field parameter sets 53A5 and 53A6. *Journal of Computational Chemistry, 25*: 1656-1676.

Patel, S. (2017). Method of isolation of pure culture. Sunandeep, Vidyapeeth, Vadodasa, Guiyarat, India.

Sandle, T. (2017). Sterilization of microbiological culture media. Institute of Validation Technology.

Trott, O. and Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry, 31*: 455-461.

Udochukwu, U., Omeje, F. I., Uloma, I. S. F. and Osiewe, D. (2015). Phytochemical analysis of *Vernonia amygdalina* and *Ocimum gratissimum* extracts and their antibacterial activities on some drug resistant bacteria. *American Journal of Research Communications, 3*(5): 225-235.

Xiao-Nan, Y., Imran, K. and Sun, C. K. (2015). Chemical composition, mechanism of antibacterial action and antioxidant activity of leaf of essential oil of *Forsythia koreana* deciduous shrub. *Asian Pacific Journal of Tropical Medicine, 8*: 604-700.

Zubairu, A. Y., Mukhtaie, M., Saidu, I., Ibrahim, Z., Isah, S., Garga, M. A. and Kebbi, H. S. (2019). Antibacterial activity of methanolic extract of bitter leaf (*Vernonia amygdalina*) from various component fractions using column chromatography. *GSC Biological and Pharmaceutical Sciences, 7*(2): 16-21.