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Toxicological Activity of the Methanolic Leaf Extract of Some Medicinal Plants Used in Sokoto Township and Environs

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Authors' contributions

This work was carried out in collaboration among all authors. Author ZAS performed all the experiments. Authors UKM, AID and AM supervised the work. Authors AM, UKM and AID helped with the literature search. Authors AM, ZAS and ASB manuscript drafting and authors MB, AUI and MG performed editing and statistical analyses. All authors read and approved the final manuscript.

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ABSTRACT

Inspite of the availability of different antiseptic and antibiotics in most localities in some parts of the world, there is still a number of information on the usage of some local plants in various kinds of treatments of different ill-health conditions. Leaves of *Ocimum basilicum, Leptadania hastata* and *Momordica balsamina* are locally used by traditional birth attendants at pre and post-partum periods. The present study investigates the phytochemical compositions and toxicity of the leaf extracts of the plants against isolates of *Listeria monocytogenes*. Standard microbiological techniques and polymerase chain reaction was used to isolate and identify the bacteria. Phytochemical analysis was done and cytotoxicity of the extracts at different concentrations (MBC, OBC and LHC) were determined using human erythrocytes. Results of the phytochemical analysis revealed the presence of tannins, flavonoids, carbohydrates, alkaloids, terpinoids and glycosides in the studied extracts.

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Toxicity to erythrocytes, expressed as percentage hemolysis of only 17.27% (MBC_1) was seen in one of the plants; *M. balsamina*. Similarly, the other extracts exhibited minimal toxicity to human erythrocytes (LHC_1= 15.45%; OBC_1= 7.6%). It was concluded that all the plant leaf extracts are safe for human consumption. Studies on the preparation, effective doses and side effects of these extracts *in vivo* are hereby recommended.

Keywords: Toxicology; Ocimum basilicum; Leptadania hastata and Momordica balsamina; medicinal plants; Sokoto Township.

1. INTRODUCTION

Many of the plant materials used in traditional medicine are readily available in rural areas. This made traditional medicine relatively cheaper than modern medicine [1,2]. It was estimated that over sixty per cent of Nigerian rural population depends on traditional medicine for their health care needs [3]. However, there still exist a vast number of tropical trees with tremendous medicinal potentials but with no empirical proof to support claims of efficacy [4,5].

Momordica balsamina from the Family Cucurbitaceae (called *Garahuni* in Hausa) is a plant widely used as an anti-inflammatory, treatment of stomach ache and the leaves as breast milk stimulant [6]. *Leptadenia hastata* Pers. Family name Asclepiadaceae, commonly known in Hausa as *Yadiya* is also a traditional plant used by indigenous traditional birth attendants at the third trimester of pregnancy. The genus *Ocimum* comprises more than 150 species and is considered as one of the largest genera of the Lamiaceae family [7]. *Ocimum basilicum* (sweet basil) known in Hausa as *Dodoya*, is an annual herb which grows in several regions all over the world. The plant is widely used in food, oral care products and the essential oil of the plant is also used as perfumery [8,9]. Koba *et al.* [10], reported *O. basilicum* and *O. gratissimum* as potential insecticides.

The emergence of drug-resistant infectious diseases is reversing the medical advances made in the last sixty (60) years [11]. Many important drugs of choice for the treatment of common infectious diseases are increasingly becoming limited, more expensive and even non-existent in some cases [12]. These underscore the need to develop new antimicrobial drugs from alternative sources [13,14]. Plant materials have been used widely in the production of drugs constituting about fifty per cent (50%) of pharmaceuticals, yet their antimicrobial potentials have not been well utilized [15].

In many African settings, traditional birth attendants use various herbs to cure pre-partum/post-partum conditions. However, there are few or none published reports, especially in the study area, about the effectiveness of these products. It is likely that some of these herbal products have some antimicrobial properties [16] against agents like *Listeria monocytogenes*, a bacterium that has been implicated in infections associated with pregnancy. This justifies the current effort to investigate the traditional claims of medicinal efficacy and the need to investigate and try to establish the antimicrobial properties of medicinal plants using approved scientific methods. This study is aimed to determine the phytochemical composition and toxicity effects of the leaf extracts of (*Ocimum basilicum*, *Leptadania hastata* and *Momordica balsamina*) used by traditional birth attendants in Sokoto.

2. MATERIALS AND METHODS

2.1 Identification of Plant Materials

The plant materials (leaves) were collected from farmlands and local markets within Sokoto township and environs, Nigeria. The plants were collected into clean polythene bags and transported to the Herbarium of Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, Nigeria for proper identification.

2.1.1 Isolation of *Listeria* species isolates

Different types of vegetables which include onions, cabbages, tomatoes and lettuce were obtained from five different selling points at Kasuwar daji, Sokoto. Each specimen was put in separate plastic bag and transported to the Microbiology Department Laboratory UDUS for analysis.

2.2 Morphological and Biochemical Characteristics of *Listeria* species

Listeria spp cultures were enriched and subcultured according to the procedure

previously described by Navas *et al*., [17]. *Listeria monocytogenes* was confirmed by its morphology and gram staining was performed in accordance to the procedure as documented by Koneman, [18]. Active motility of the bacteria was determined using the method and characteristics described elsewhere. Some biochemical tests (Catalase and Oxidase) were performed using the procedure described by Cheesborough, [19].

2.3 Molecular Identification of *Listeria* species

DNA extraction was done as previously documented [20,21].

2.3.1 Amplification of 16S rRNA region of the bacterial genome

Two designed primer sets of partial ribosomal DNA (16S) of *Listeria* species genome 27F **(**5'**-**AGAGTTTGATCMTGGCTCAG**-**3') and 907R (5'**-**CCGTCAATTCMTTTRAGTTT-3') were used to amplify a 956 bps target in the gene as described by David *et al*. [22]. A 25 µl reaction mix containing the following component: Qiagen, toptag PCR master mix 2.5 µl 0.2 µl, of each forward and reverse primer, 17.1 µl of nuclease free-water, 5 µl of DNA, template in a 0.2 microtube (PCR tube) up to final volume of 25 µL. One of the reaction tubes without a DNA template was used as a negative control (N) to assume absence of environmental contamination. the tubes were capped and transferred into applied Biosystem 7700 thermocycler with the following cycling conditions: denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min with a final extension at 72°C for 10 min for 35 cycles. The DNA bands were then viewed in a 1% Agarose gel [23] by illumination with UV light and images recorded by photography (Biorad imager).

2.4 Cytotoxicity by Hemolytic Activity on Human Erythrocytes

Human A+, B+ and O+ red blood cells were used for this assay. The cells, obtained from the blood transfusion unit Usmanu Danfodiyo University Teaching Hospital (UDUTH), was washed three times in 9 volumes of sterile 0.85% NaCl solution [24]. After each washing, the cells were pelleted by centrifugation at 150 x g for 5 mins and the supernatant was discarded. The final pellet was diluted 1:9 (v/v) in sterile 0.85% normal saline solution, this was followed by a 1:24 (v/v) dilution in sterile phosphate buffered saline (PBS) pH 7.0 containing 0.5 mM boric acid and 1 mM calcium chloride [25]. The red cell suspensions (1 mL of final volume) were then incubated with an aqueous solution of the extract being investigated (50–500 µg/mL in 0.85% NaCl). The tubes were then incubated for 6, 12 and 24 h at 37^{o}C [25]. During the incubation period the samples was resuspended occasionally (maximum once per h) by inversion.

Negative control of RBC suspensions in 100 µM ouabain was incubated along with the test samples [24]. Total lysis of the standard (100% lysis) erythrocytes was obtained by incubating the cells with 0.1 % (v/v) Tween 20. In order to evaluate the degree of spontaneous lysis, tubes containing only red blood cell suspension in PBS was set. For each concentration and control, the experiments were set in triplicates.

After the incubation, the cell suspensions were centrifuged at 900xg for 10 minutes [25]. The supernatant was carefully collected, paying attention not to disturb the pellet. The absorbance at 405/540 nm of supernatant was measured with a spectrophotometer. The value of absorbance of erythrocytes to be maintained exclusively in the PBS solution was used to 'zero' (blank) the spectrophotometer before reading the samples that will contain the plant extracts. The level of hemolysis was expressed as a percentage hemolysis; calculated as the ratio of the absorbance value for each sample and that for the total hemolysis [25].

2.5 Statistical Analysis

SPSS version 25.0 was used for statistical analysis, data generated was presented in the form of tables and graphs where appropriate. All measured parameters were calculated and expressed as Mean ±Standard Deviation or percentages. For all numerical values, homogeneity of variances was tested using the Bartlett's test. Probability of 0.05 ($p < 0.05$) was used as the criterion of significance.

3. RESULTS

The study determines the antimicrobial activity of *M. balsamina, O. Basilicum* and *L. hastata* leaves extracts using nine (9) isolates of *L. monocytogenes* isolated from vegetables in Sokoto metropolis. The source and biochemical reaction of each isolate included in the study are depicted in Table 1. Three of the isolates (O4,

O9 and O14) were recovered from onion samples, two from (L5 and L13) lettuce, two (T9 and T13) from tomatoes and another two isolates (C2 and C3) from cabbages. The corresponding morphologic characteristics (growth on *Listeria* agar, Gram stain and motility) and some biochemical reactions (catalase and oxidase) for each of the isolates are shown in the same table.

Following PCR amplification of the extracted DNA for molecular confirmation of *Listeria monocytogenes* from cultures of the vegetable samples, the results revealed a 956 bp gene from the 16S ribosomal subunit which was obtained via electrophoresis of the PCR product. Absence of bands in the negative control sample (N) is an indication that there was no contamination in the reaction (Fig. 1).

The phytochemical analysis revealed the presence of tannins, saponins, flavonoids, carbohydrates, alkaloids, phenols, terpinoids and glycosides in all the studied extracts. Cholesterol, was found to be present in all the extracts except in *L. hastata*. However, phlabotannins tested positive only for the *O. basilicum* leaf extract (Table 2). Table 3 showed the toxicity of different concentrations of the extracts against human erythrocytes. The percentage haemolysis was determined against the Optical Density (OD) of erythrocytes lysed in distilled water (100% hemolysis with OD of 1.83).

Table 1. Morphological and biochemical identification of *L. monocytogenes* from vegetables

S/N	Isolate	Gram rxn	Shape	Motility	Catalase	Oxidase	Suspected Organism
1	O4	+	R	Motile	+	-	*Listeria* sp
2	O9	+	R	Motile	+	-	*Listeria* sp
3	C3	+	R	Motile	+	-	*Listeria* sp
4	L5	+	R	Motile	+	-	*Listeria* sp
5	T13	+	R	Motile	+	-	*Listeria* sp
6	L13	+	R	Motile	+	-	*Listeria* sp
7	C2	+	R	Motile	+	-	*Listeria* sp
8	O14	+	R	Motile	+	-	*Listeria* sp
9	T9	+	R	Motile	+	-	*Listeria* sp

KEY: isolates from this point onwards were identified by an annotation that includes a single letter and a number; the letter represents the first alphabet in name of the vegetable from which it was isolated while the number is a serial number that identified a vegetable from a collection. Thus; O4 = Onion 4th isolate; O9 = Onion 9th isolate; C3 = Cabbage 3rd isolate; L5 = Lettuce 5th isolate; T13 = Tomato 13th isolate; L13 = Lettuce 13th isolate; C2 = Cabbage 2nd isolate; O14 = Onion 14th isolate; T9 = Tomato 9th isolate; R=Rod;

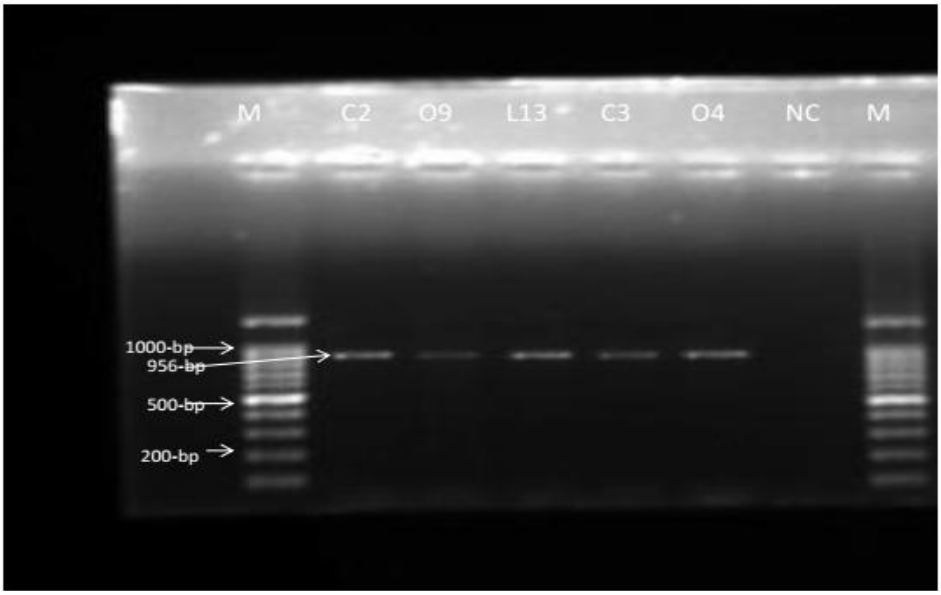

Fig. 1. Agarose gel electrophoresis pattern of 16S ribosomal subunits specific for *Listeria monocytogenes*. M=DNA Ladder; C2, O9, L13, C3, O4 =samples; NC= Negative control

Table 2. Preliminary screening for Phytochemicals composition of some plants used by traditional birth attendants

Phytochemicals	*Mormodica balsamina*	*Ocimum basilicum*	*Leptadania hastata*
Tannins	+	+	+
Phlabotannins	-	+	-
Saponins	+	+	+
Flavonoids	+	+	+
Cardiac glycosides	+	+	+
Carbohydrates	+	+	+
Cholesterol	+	+	-
Terpinoids	+	+	+
Alkaloids	+	+	+
Phenols	+	+	+
Glycosides	+	+	+

Table 3. Toxicity of different concentration of the plant extracts against human erythrocytes

Conc.	OD	Haemolysis (%)
OBC_1	0.139	7.6
OBC_2	0.118	6.44
OBC_3	0.034	1.86
MBC_1	0.316	17.27
MBC_2	0.262	14.3
MBC_3	0.239	13.06
LHC_1	0.282	15.45
LHC_2	0.081	4.43
LHC_3	0.015	0.82

Key: MBC_1 = Momordica balsamina concentration 1, OBC_1 = Ocimum basilicum concentration 1 and LHC_1 = Leptadania hastate concentration 1

4. DISCUSSION

Current global interest in the use of eco-friendly and economical natural resources triggered the wide application of highly acclaimed medicinal plants and especially by traditional healers [26] which also instigate the investigation of plants use and by the traditional birth attendants at the third trimester pregnancy period. In fact, the results showed that in all the three samples studied, the qualitative phytochemical analysis of the crude extract appears to be marginally different with one or two bioactive compounds absent and/or present with a varied concentration as have been reported previously [27]. Some of these phytochemicals may be believed to be responsible for high therapeutic potency, including antibacterial, antitoxic etc [28].

Basically, in all the samples investigated via qualitative phytochemical analysis, all the bioactive chemicals were present in *O. basilicum*, which portrays their unique antioxidant functionality as well as medicinal benefits which may include maintaining inflammation balance, reducing the risk of certain cancers and promoting cardiovascular, neurocognitive and bone health in humans [29]. These among other things may be the reasons why the traditional birth attendants often recommend the use of *O. basilicum* at the third trimester of pregnancy; even though the bioavailability in humans can be affected by various factors such as food matrix, interactions with other nutrients, site of absorption and their metabolism [29].

Phytochemical screening of *M. balsamina* also reveals the presence of tannins, saponins, flavonoids, carbohydrates, terpinoids, glycosides, flavonoids, Cholesterol, alkaloids and phenols with the exception of phlabotannins. These compounds have been reported in the leaves of *M. balsamina* in many studies [30-32].

Phytochemical screening of *Leptadania hastata* has indicated the presence of tannins, saponins, flavonoids, carbohydrates, terpinoids, glycosides, and flavonoids, with the exception of Cholesterol, and phlabotannins. A similar study conducted by Bello *et al.* [33] on *L. hastata* leaves has also indicated the presence of phenolic glycosides, tannins, flavonoids, proanthocyanidins, alkaloids and saponins. Decoction of the leaves of *L. hastata* with the bark of *Erythrina senegalensis* is either taken orally or used as a medicinal bath to treat onchocercosis in Mali [34] In Chad, the roots are used to treat scabies [35]. In Burkina Faso, locally it is used for sexual potency (chewing leaves), trypanosomosis (decoction of leaves), skin diseases and wound-healing (application of latex) [36].

In Senegal, the leaves have been reportedly used for purgative as well as lactation and as a

purgative [37,38]. It is also reported that Senegalese healers use the *L. hastata* for prostate and rheumatism complaints [39]. Ibrahim *et al*. [40], has reported in Nigeria that this plant is commonly used in Hausa-speaking communities as a spice and used in sauces. Similarly, in the same country, local healers use the plant for hypertension, catarrh and skin diseases [41] and now that traditional birth attendant are using it during the third trimester period of pregnancy.

Similarly, the presence of Flavanoids in all the samples was not surprising because flavonoids are the colouring matter of plants that are commonly found in the leaves and flowers [42]. Tannins is actually expected and found in all the three plants because they are present in higher plant; tannins are responsible for the reddish colour of sliced stem and root barks of woodiest plant [4]. Alkaloids were found present in all the leaves of the samples being one of the predominantly medicinal compounds. That its usage cut across numerous ailments ranging from simple analgesic to treatment of malaria, hypertension etc. some of them are known to be very poisonous [43]. The reputation of alkaloids as potent medicines called for attempts to extracts, isolate and characterize the alkaloids present in this plant. This isn't different from what has been previously anticipated [44, 45].

Furthermore, the toxicity of different concentrations of the extracts against human erythrocytes was also evaluated and presented. The percentage haemolysis was determined against the Optical Density (OD) of erythrocytes lysed in distilled water (100% hemolysis with OD of 1.83) which indicated the safety of the use of *O. basilicum, M. balsamina* and *L. hasttata*. A similar result was obtained from Tamboura *et al*. [36], who conducted their experiments by the means of male albino mice using concentrations 1000-2000 mg/kg body weight of *L. hastata* aqueous extract (leaves and stems). The mice were injected with the extract intraperitoneally and were observed during 48 to 72 hours; and *L. hastata* is thus declared safe to use due to its high LD quotient value of 0.78.

Also, a study to determine acute and subchronic toxicity of Wistar rats [46] was conducted in Iran. Based on common classification of the relative toxicity of chemicals, acute toxicity results indicated that *O. basilicum* hydroalcoholic extract was classified as a non-toxic substance because the oral LD50 was greater than 5000 mg/kg. However, the study reported that the subchronic oral administration of *O. basilicum* to Wistar rats did not cause death or any abnormal dose dependent changes in biochemical and liver histopathological parameters. But Hamid *et al*. [46], mentioned that a number of significant hematological changes were associated with the subchronic oral administration of *O. basilicum* to Wistar rats. Moreover, the report asserted that, hematologic system is a possible target organ for both sexes although the exact mechanisms are unclear but the report concluded that that the risk of oral toxicity of *O. basilicum* on mammals is not negligible.

5. CONCLUSION

The study highlights the efficacy of " traditional medicine " which is an ancient tradition, used in some parts of Nigeria. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable. Qualitative phytochemical screening of the leaf extracts of the three plants have shown the chemical composition of the plants. Toxicity tests results revealed that the leaf extracts exhibited minimal toxicity to human erythrocytes which signifies the safety of the plants for human consumption.

6. RECOMMENDATIONS

Studies to determine effective doses of the plants' preparations and side effects of these extracts (*in vivo*) are warranted.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mahomoodally MF. Traditional medicines in Africa: An appraisal of ten potent African medicinal plants %j evidence-based complementary and alternative medicine. 2013;14.
2. Umair M, Altaf M, Abbasi AM. An ethnobotanical survey of indigenous medicinal plants in Hafizabad district, Punjab-Pakistan. PLoS One. 2017;12:e 0177912.
3. Ajoku GA, Ibrahim K, Enweren NM, Odoma LE, Inyang KS. Preliminary Phytochemical Screening of Detarium senegalensis Gmelin (cealpiniacae) against some isolates of Tropical cultured collection (ATCC). Nigerian Journal of Botany. 2005;18:289-293.
4. Akindahunsi AA, Salawu SO. Phytochemical Screening and Nutrient-antinutrient composition of selected Tropical green leafy vegetables. African Journal of Biotechnology. 2005;4(6):49-501.
5. Petrovska BB. Historical Review of Medicinal Plants' Usage. Pharmacognosy Reviews. 2012; 6(11):1-5.
6. Abubakar MS, Musa AM, Ahmed A, Husaini IM. The perception and practice of traditional medicine in the treatment of cancers and inflammations by the Hausa and Fulani tribes of northern Nigeria. Journal of Ethnopharmacology. 2007;111 :625–62.
7. Hameed IH, Hamza LF, Kamal SA. Analysis of bioactive chemical compounds of *Aspergillus niger* by using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. Journal of Pharmacognosy and Phytotherapy. 2015;7(8):132-163.
8. Chiang LC. Antiviral activities of extracts and selected pure constituents of *Ocimum basilicum*. Clinical and Experimental Pharmacology and Physiology. 2005;32(10):811-816.
9. Ali B, Al-Wabel NA, Shams S, Ahamad A, Khan SA, Anwar F. Essential oils used in aromatherapy: A systemic review. Asian Pacific Journal of Tropical Biomedicine. 2015;5(8): 601-611.
10. Koba K, Poutouli PW, Raynaud C, Chaumont JP, Sanda K. Chemical composition and antimicrobial properites of different basil essential oils chemotyypes from Togo. Bangladesh Journal of Pharmacology. 2009;4:1–8.
11. Jasovský D, Littmann J, Zorzet A, Cars O. Antimicrobial Resistance-a Threat to the World's Sustainable Development. Upsala Journal of Medical Sciences. 2016;121(3):159-164.
12. Li B, Webster TJ. Bacteria Antibiotic Resistance: New Challenges and Opportunities for Implant-Associated Orthopedic Infections. Journal of orthopaedic research: Official Publication of the Orthopaedic Research Society. 2018;36(1):22-32.
13. Shah PM. The need for new therapeutic agents: what is in the pipeline? Clinical Microbiology and Infection. 2005;11(3):36-42.
14. Cheesman MJ, Ilanko A, Blonk B, Cock IE. Developing New Antimicrobial Therapies: Are Synergistic Combinations of Plant Extracts/Compounds with Conventional Antibiotics the Solution? Pharmacognosy Reviews. 2017;11(22):57-72.
15. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Frontiers in Pharmacology. 2014;4:177-177.
16. Phumthum M, Srithi K, Inta A, Junsongduang A, Tangjitman K, Pongamornkul W, Trisonthi C, Balslev H. Ethnomedicinal plant diversity in Thailand. J. Ethnopharmacol. 2018; 214:90–98.
17. Navas J, Ortiz S, Lopez P, Lopez V, Martinez-Suarez JV. Different Enrichment Procedures for Recovery of *Listeria monocytogenes* from Raw Chicken Samples Can Affect the Results of Detection (by Chromogenic Plating or Real-Time Pcr) and Lineage or Strain Identification. Journal of Food Protection. 2007;70(12):2851-2854.
18. Koneman EW. Koneman's color atlas and textbook of diagnostic microbiology (color atlas and textbook of diagnostic microbiology) 6th Edition. Lippincott Williams and Wilkins; 2005.
19. Cheesbrough M. District Laboratory Practice in Tropical Countries, part two, 2nd edition. Cambridge University Press, Cambridge, England. 2010;62-70.

20. Wu C, Dasgupta A, Shen L, Bell-Pedersen D, Sachs MS. The cell free protein synthesis system from the model filamentous fungus Neurospora crassa. Methods (San Diego, Calif.). 2018;137: 11–19.
21. Hwang S, Alhatlani B, Arias A, Caddy SL, Christodoulou C, Cunha JB, Wobus CE. Murine norovirus: propagation, quantification, and genetic manipulation. Current Protocols in Microbiology, 2014;33:15K.2.1–15K.2.61.
22. David M, Marcia B, Guillermina R, Lisette L, Aldo G, Roberto M, Vidal MV. Molecular epidemiology and genetic diversity of *Listeria monocytogenes* isolates from a wide variety of ready-to-eat foods and their relationship to clinical strains from listeriosis outbreaks in Chile. Front Microbiol. 2015;6:384.
23. Law JW, Ab Mutalib NS, Chan KG, Lee LH. An insight into the isolation, enumeration, and molecular detection of Listeria monocytogenes in food. Front Microbiol. 2015;3(6):1227.
24. Róka E, Ujhelyi Z, Deli M, Bocsik A, Fenyvesi É, Szente L, Bácskay IK. Evaluation of the Cytotoxicity of α-Cyclodextrin Derivatives on the Caco-2 Cell Line and Human Erythrocytes. Molecules (Basel, Switzerland), 2015;20(11):20269–20285. DOI:10.3390/molecules201119694.
25. Taniyama S, Arakawa O, Terada M, Nishio S, Takatani T, Mahmud Y, Noguchi T. Ostreopsis sp., a possible origin of palytoxin (PTX) in parrotfish Scarus Ovifrons. Toxicon. 2003;42(1):29-33.
26. Yuvenalis M. Mbuni, Shengwei Wang, Brian N. Mwangi, Ndungu J. Mbari, Paul M. Musili, Nyamolo O. Walter, Guangwan Hu, Yadong Zhou, and Qingfeng Wang. Medicinal Plants and Their Traditional Uses in Local Communities around Cherangani Hills, Western Kenya. Plants, 2020;9:331; doi:10.3390/plants9030331
27. Ansari I, Patil DT. A brief review on phytochemical and pharmacological profile of Carissa spinarum. Asian J. Pharm. Clin. Res. 2018;11:12–18.
28. Rakesh KS. Uses of Medicinal Plants in Traditional and Modern Medicine, International Journal of Engineering Research & Technology (IJERT). 2019;8(8):
29. Yeum KJ, Russel RM. Biological functions of Plant Figment Phytochemicals in Humans In. Laher I. (eds) System Biology of Free Radicals and Antioxidants. Springer, Berlin, Heildelberg;2014.
30. Akinniyi JA, Sultanbawa MUA, Manawaku D. In: The State of Medicinal Plant Research in Nigeria. University Press: lbadan Nigeria. 1986;154-165.
31. Karumi Y, Bobboi A. Hypoglycemic effects of Balsam Apple (*Momordica balsamina* Linn) in Alloxan diabetic male rabbits. Biochemistry. 1999;9:795-808.
32. Shettima YATM, Karumi Y, Sodipo OA. Phytochemical and Antidiarrhoeal Properties of Methanol root Extract of Guiera senegalensis J.F .Gmel. International Research Journal of Pharmacy. 2012;3(11):61-65.
33. Bello A, Aliero AA, Saidu Y, Muhammad S. Phytochemical Screening, Polyphenolic Content and Alpha-Glucosidase Inhibitory Potential of *Leptadenia hastata* (Pers.) Decne. Nigerian Journal of Basic and Applied Science. 2011;19:181- 186.
34. Togola A, Austarheim I, Theïs A, Diallo D, Paulsen BS. Ethnopharmacological uses of Erythrina senegalensis: A comparison of three areas in Mali, and a link between traditional knowledge and modern biological science. Journal of Ethnobiology and Ethnomedicine. 2008;4:6.
35. Betti JL, Yemefa'a SRM, Tarla NF. Contribution to the knowledge of non-wood forest products of the far north region of Cameroon: Medicinal plants sold in the Kousséri market. Journal of Ecology and the Natural Environment. 2011;3(7):241-254.
36. Tamboura HH, Bayala B,, Lompo M, Guissou IP, Sawadogo L. Ecological distribution, morphological characteristics and acute toxicity of aqueous extracts of *Holarrhena floribunda* (G. Don) Durand & Schinz, *Leptadenia hastata* (Pers.) Decne and Cassia sieberiana (dc) used by veterinary healers in Burkina Faso. African Journal of Traditional Complementary and Alternative Medicine. 2005;2:13-24.
37. Kerharo J, Adam JG. The traditional Senegalese pharmacopoeia: Medicinal and poisonous plants. Vigot brother's edition, Paris; 1974.
38. Arbonnier M. Trees, shrubs and vines dry areas of West Africa; 2000.
39. Mathieu G, Meissa D. Traditional leafy vegetables in Senegal: diversity and medicinal uses. African Journal of Traditional, Complementary and

Alternative Medicines. 2007;10:469-475.
40. Ibrahim HA, Ali GY, Halliru SN, Usaini S, Abdullahi II. Ethnobotanical Survey of the Wild Edible Food Plants Consumption among Local Communities in Kano State, North-Western, Nigeria. International Journal of Science and Technology. 2012;2:713-717.
41. Dambatta SH, Aliyu BS. A survey of major ethnomedicinal plants of Kano North Nigeria, their knowledge and uses by traditional healers. Bayero Journal of Pure and Applied Sciences. 2011; 4(2):28-34.
42. Njoku PC, Ezeibis AU. Phytochemical and Elemental Analysis of Helian thus Annus and its uses as blood Clothery agent, Journal of Chemical Society of Nigeria. 2007;32 (2):128-132.
43. Stray F. The Natural Guide to Medicinal Herbs and Plants. Tiger book international, London, 1998;12-16.
44. Odongo E, Mungai N, Mutai P, Karumi E, Mwangi J, Omale J. Ethnobotanical survey of the medicinal plants used in Kakamega County, Western Kenya. Appl. Med. Res. 2018;4:22–40.
45. Kigen G, Kamuren Z, Njiru E, Wanjohi B, Kipkore W. Ethnomedical Survey of the Plants Used by Traditional Healers in Narok County, Kenya. Complement. Altern. Med. 2019;8976937.
46. Hamid R, Leila H, Soghra M, Mohammad K, Majid A, Farahnaz T. Safety Assessment of Ocimum basilicum Hydroalcoholic Extract in Wistar Rats: Acute and Subchronic Toxicity Studies. Iranian Journal of basic Medical Sciences. 2012;15(1):652.

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