

TOXICOLOGICAL ACTIVITY OF THE METHANOLIC LEAF EXTRACT OF SOME MEDICINAL PLANTS USED IN SOKOTO TOWNSHIP AND ENVIRONS

Abstract

In spite 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 were determined using human erythrocytes. Results of the phytochemical analysis revealed the presence of tannins, flavonoids, carbohydrates, alkaloids, terpenoids and glycosides in the studied extracts. Toxicity to erythrocytes, expressed as percentage hemolysis (17.27%) was seen in one of the plants *M. balsamina*. Similarly, the other extracts exhibited minimal toxicity to human erythrocytes. 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.

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 (Mahomoodally, 2013). It was estimated that over sixty per cent of Nigerian rural population depends on traditional medicine for their health care needs (Ajoku *et al.*, 2005). However, there still exist a vast number of tropical trees with tremendous medicinal potentials but with no empirical proof to support claims of efficacy (Akindahunsi and Salawu, 2005; Petrovska, 2012).

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 (Abubakar *et al.*, 2007). *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 (Hameed *et al.*, 2015). *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 (Chiang, 2005; Ali *et al.*, 2015). Koba *et al.* (2009), 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 (Jasovský *et al.*, 2016). 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 (Li and Webster, 2018). These underscore the need to develop new antimicrobial drugs from alternative sources (Shah, 2005; Cheesman *et al.*, 2017). 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 (Ekor, 2014).

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 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.

Materials and Methods

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.

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.

Morphological and Biochemical Characteristics of *Listeria* species

Listeria spp cultures were enriched and subcultured according to the procedure previously described by Navas *et al.*, (2007). *Listeria monocytogenes* was confirmed by its morphology and gram staining was performed in accordance to the procedure as documented by Koneman, (2005). 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, (2010).

Molecular Identification of *Listeria* species

DNA extraction was done as previously documented (Wu *et al.*, 2018; Hwang *et al.*, 2014).

Amplification of 16S rRNA region of the bacterial genome

Two designed primer sets were used to amplify 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 Jiang *et al.* (2006). 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 (Murakami *et al.*, 1991) by illumination with UV light and images recorded by photography (Biorad imager).

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 (Róka *et al.*, 2015). 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 (Taniyama *et al.*, 2003). 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°C (Taniyama *et al.*, 2003). 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 (Róka *et al.*, 2015). 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 (Taniyama *et al.*, 2003). 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 (Taniyama *et al.*, 2003).

- **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.

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 (Figure 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	+	-	<i>Listeria</i> sp
2	O9	+	R	Motile	+	-	<i>Listeria</i> sp
3	C3	+	R	Motile	+	-	<i>Listeria</i> sp
4	L5	+	R	Motile	+	-	<i>Listeria</i> sp
5	T13	+	R	Motile	+	-	<i>Listeria</i> sp
6	L13	+	R	Motile	+	-	<i>Listeria</i> sp
7	C2	+	R	Motile	+	-	<i>Listeria</i> sp
8	O14	+	R	Motile	+	-	<i>Listeria</i> sp
9	T9	+	R	Motile	+	-	<i>Listeria</i> 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;

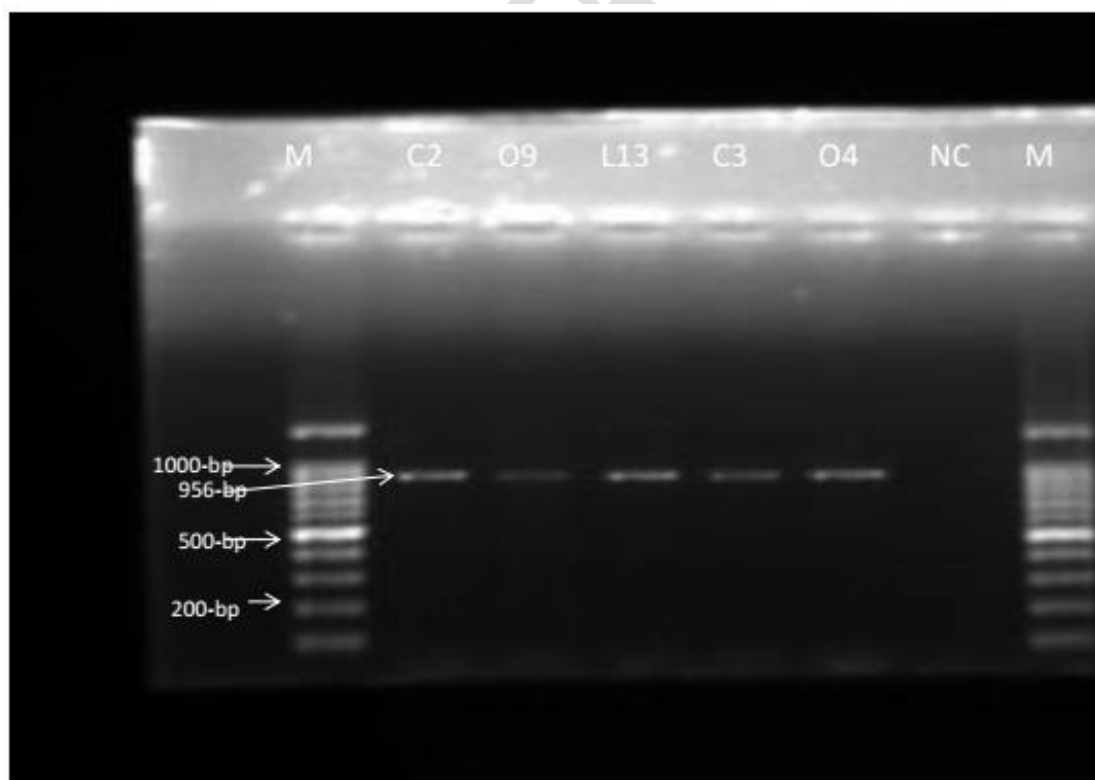


Figure 1: Agarose gel electrophoresis pattern of 16S ribosomal subunits specific for *Listeria monocytogenes*. M=DNA Ladder; C2, O9, L13, C3, O4 =samples; NC= Negative control.

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

Phytochemicals	<i>Mormodica balsamina</i>	<i>Ocimum basilicum</i>	<i>Leptadania hastata</i>
Tannins	+	+	+
Phlabetannins	-	+	-
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:

MBC₁ = *Momordica balsamina* concentration 1, OBC₁ = *Ocimum basilicum* concentration 1 and LHC₁ = *Leptadania hastate* concentration 1

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 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 of tannins, saponins, flavonoids, carbohydrates, alkaloids, phenols, terpinoids, glycosides, cardiac glycosides, cholesterol and phlobatannins. Some of these phytochemicals may be believed to be responsible for high therapeutic potency (including antibacterial, antitoxic etc.).

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 (Yeum and Russell, 2014). 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 (Yeum and Russell, 2014).

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 (Akinniyi *et al.*, 1986; Karumi and Bobbi, 1999; Shettima *et al.*, 2001).

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.* (2011) 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 (Togola *et al.*, 2008) In Chad, the roots are used to treat scabies (Betti *et al.*, 2011). In Burkina Faso, locally it is used for sexual potency (chewing leaves), trypanosomosis (decoction of leaves), skin diseases and wound-healing (application of latex) (Tamboura *et al.*, 2005).

In Senegal, the leaves have been reportedly used for purgative as well as lactation and as a purgative (Kerharo and Adam, 1974; Arbonnier, 2000). It is also reported that Senegalese healers use the *L. hastata* for prostate and rheumatism complaints (Mathieu and Meissa, 2007). Ibrahim *et al.* (2012), 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 (Danbatta and Aliyu, 2011) 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 (Njoku and Ezeibis, 2007). 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 (Akindahunsi and Salawu, 2005). 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 (Stray, 1998). The reputation of alkaloids as potent medicines called for attempts to extracts, isolate and characterize the alkaloids present in this plant.

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. hastata*. A similar result was obtained from Tamboura *et al.* (2005), 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 (Hamid *et al.*, 2011) 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.* (2011), 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.

Conclusion

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.

Recommendations

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

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Authors Contributions

ZAS performed all the experiments. UKM, AID and AM supervised the work. AM, UKM and AID helped with the literature search. AM, ZAS and ASB manuscript drafting and MB, AUI and MG performed editing and statistical analyses. All authors read and approved the final manuscript.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

NOTE:

The study highlights the efficacy of " traditional medicine " which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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