PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTILISTERIAL ATTRIBUTES OF DIFFERENT AQUEOUS AND ETHANOLIC LEAF EXTRACTS

Daniel O. Ebakota[1+], Onilude A. Abiodun[2] and Obayagbona Omoregbe Nosa[3]

[1]Department of Biological Sciences, Microbiology Option, Benson Idahosa University, Benin City, Nigeria

[2]Department of Microbiology, University of Ibadan, Ibadan, Nigeria

[3]Department of Environmental Management and Toxicology, University of Benin, Benin City, Nigeria
[+]Corresponding author email address: eoziengbe@biu.edu.ng +2348038554711

Introduction

Plants produce a vast diversity of secondary metabolites most of which are phytochemicals that have potential use in the pharmaceutical industry for new drug development purposes. Phytochemicals are naturally-occurring bioactive plant compounds that act as a natural defence system for the host plant and also provide colour, aroma, and flavor [1]. Some phytochemicals have been shown to possess antimicrobial properties and these include terpenoids, essential oils, alkaloids, lectins, polypeptides, polyacetylenes, and phenolics, of which phenolics can be further divided into phenolic acids, flavonoids, quinones, tannins, coumarins, and simple phenols [2].

A variety of herbs and spices have been tested for their efficacy in suppressing the growth of *L. monocytogenes* in culture media. Plant extracts that have shown to exhibit antilisterial activity include: hop extracts, eugenol [3], pimento leaf, horseradish distillates [4], rosemary, cloves[5], cinnamic acid, furanocoumarins and carvacol [6]. *Garcinia kola*, *Moringa olifera*, *Origanum syriacum*, *Majorana hortensis*, *Rosmarinus officinalis*, *Cymbopogon citratus*, *Thymus vulgaris*, *Achillea millefolium*, *Calendula officinales* and *Artemisia annua* have also been reported to exhibit antilisterial activity [7,8]. Different commercial samples of plant essential oils and different varieties of the same herbs may exhibit differences in antilisterial potency because of varying amounts of phytochemicals.

Plant extracts found to be effective against *Listeria* spp. in meat include; rosemary in ready-to-eat pork liver sausage, horseradish distillates on roast beef, and eugenol and pimento leaf on refrigerated cooked beef. It was observed that *L. monocytogenes* was usually less sensitive to these extracts in meat (compared to culture media) [9]. In addition, the antimicrobial property of medicinal plants may differ depending on the form of added plants, such as fresh, dried, or extracted forms.

Daniel *et al*.[10] reported the isolation of about 90 multidrug resistant *L. monocytogenes* strains from about 411 ready-to-eat (RTE) foods sold at several locations in southern Nigeria. The authors also documented that all the food borne *L. monocytogenes* strains exhibited resistance against amoxicillin, cloxacillin, augumentin and ceftazidime. The aim of this study was to evaluate the phytochemical contents and *in vitro* antilisterial attributes of prepared extracts from several tropical plants against previously documented RTE associated multi antibiotic resistant *L. monocytogenes* strains. The examined aqueous and ethanolic extracts were derived from *Psidium guajava*(guava), *Zingiber officinale*(Ginger), *Dacryodes edulis* (African pear),*Citrus aurantifolia*(Lime),*Funtumia elastica* (silkrubber), *Vernonia amygdalina* (Bitter leaf),*Cassia alata*and *Moringa oleifera*(horseradish tree).

Materials and methods

Sources of medicinal plants

Leaves of the medicinal plants; *P. guajuva*, *Z. officinale*, *D. edulis*, *C. aurantifolia*, *F. elastica*, *V. amygdalina*, *C. alata*and *M. oleifera* and were obtained from farms at Ekpoma with geographical coordinate's 6°45′ North 6°08 ′East Edo state, Nigeria. The identity of the respective plant leaves was confirmed by Mr. O. Eguagie of the Department of Plant Biology and Biotechnology, University of Benin, Edo state, Nigeria. The leaves of these plants were dried at room temperature for about three (3) weeks then they were pulverized and stored in clean polythene bag until required.

Preparation of aqueous and ethanolic plant extracts

Fifty grams (50g) of each dried powdered leaves was soaked in water and ethanol for three days. At the end of the third day, the extracts were filtered and the filtrate was concentrated using rotary evaporator at 40°C. After complete evaporation, the extract was preserved aseptically at 4ºC until required.

Phytochemical screening

The ethanolic and aqueous extracts of the plants were screened qualitatively for the presence of flavonoids, cardiac glycosides, reducing sugars, tannins, saponins, terpenoids, alkaloids, phenolics, resins and steroids using procedures as described by Harborne [11] , Trease and Evans[12] and, Obadoni and Ochuko [13].

Source and standardization of RTE associated L. monocytogenes strains

Three (3) *L. monocytogenes* strains earlier isolated and identified from RTE foods by Daniel *et al.* [10] were utilized in this study. The authors had also coded the respective *L. monocytogenes* strains as; LMSN 70, LMEW 94 and LMMP 104 respectively. Procedures as described by Azu and Onyeagba,[14] and Asowata *et al.* [15] and were adapted in the standardization of the Listerial test pure cultures. The three (3) *L. monocytogenes* strains were sub-cultured on freshly prepared Nutrient agar plates and incubated for 24 h respectively. Portions of the streaked Listerial colonies were transferred into test tubes containing 8ml of sterile nutrient broth and incubated for 12h at 37 ^{0}C. The growth of bacterial suspension obtained was compared to that of freshly prepared Barium sulphate opacity standard {0.5 ml of 1% Barium in Chloride to 99.5

DOI: 10.5281/zenodo.3469444

ml of 1% H_2SO_4 (0.36 Normal) with the aid of a Spectrophotometer at 600nm (Thermoscientific genesys UV-Visible spectrophotometer). The turbidity was adjusted by adding more sterile nutrient broth to match 0.5 McFarland standards (10^6 cfu/ml).

Preparation of plant extract concentrate

Varying concentrates; 200mg/mL, 400mg/mL, 600mg/mL, 800mg/mL and 1000mg/mL dilution of the different leaf extracts (aqueous and ethanol) was prepared by the dissolution of 0.2g, 0.4g, 0.6g, 0.8g and 1g of the respective extract in 1mL sterile distilled water.

Agar well diffusion assay

Antimicrobial activities of the aqueous and ethanolextracts of the leaves were evaluated by means of agar-well diffusion assay as described by Adeshina *et al*. [16]. Fifteen (15) mL of the molten Muller-Hinton agar was poured into sterile petri dishes. Approximately, 0.1mL of the prepared standardized *L. monocytogenes* inoculum was evenly spread with a swab stick onto the surface of the solidified agar plates. Once the plates had been aseptically dried, wells were punched into the agar with a sterile cork borer (5mm) after which 0.2mL of the prepared extracts were placed into the wells and the plates were incubated at 37°C for 24h. Antilisterial activity was evaluated by measuring the diameter zone of inhibition around the wells.

Results and discussion

The phytochemical screening of the different ethanolic plant leaf extracts is shown in Table 1. *Z. officinale* leaf extract had alkaloids, saponins, steroids, terpenoids, cardiac glycoside, reducing sugar, phenolics, resins, flavonoids, and tannins present in varying concentrations. Cardiac glycoside was absent in *D. edulis*, *C. aurantifolia* and *F. elasticaleaf* extracts whilst tannins and terpenoids was absent in *Cassia alata* and *M. oleifera* leaf extract respectively. The phytochemical screening of the different aqueous plant leaf extracts is presented in Table 2. *Z. officinale* and *M. oleifera* extracts had alkaloids, saponins, steroids, terpenoids, cardiac glycoside, reducing sugar, phenolics, resins, flavonoids, and tannins present in varying amounts. Cardiac glycoside was present in *P. guajuva*, *C. aurantifolia* and *F. elastica* extracts but absent in *D. edulis*extract. Alkaloids and tannins was absent in *C. alata* extract while steroids were absent in *F. elastica* extract.

The antilisterial activity of different ethanolic leaf extracts against the threeRTE borne *L. monocytogenes* strains is shown in Table 3. Guava leaf extract elaborated antilisterial activity against *L. monocytogenes* LMSN70 with inhibitory growth zones of 30 mm± 2.00, 45 mm ± 14.12, 50 mm ± 7.07, 57 mm± 0.00, 60 mm ± 0.00, and 65mm ± 0.00 at 100 mg/mL, 200 mg/mL, 400 mg/mL, 600 mg/mL, 800 mg/mL and 1000 mg/mL respectively. Ginger leaf ethanolic extract did not any antilisterial activities at concentration less than 600mg/ml but had 40 ± 0.00, 25 ± 4.23 and 20 ± 7.23 at 1000mg/mL for *L. monocytogenes* LMSN70, LMEW94, LMMP104 respectively. Bitter leaves had antilisterial activity against *L. monocytogenes* LMEW94 with 35 ± 0.00, 50 ± 0.00, 60 ±2.33, 64 ± 2.23, 70 ± 0.00, and 35 ± 0.00 at 100 mg/mL, 200 mg/mL, 400 mg/mL, 600 mg/mL, 800 mg/mL and 1000 mg/mL respectively. Moringa ethanol leaf extract did not exhibit any antilisterialactivity against the exposed RTE borne L. *monocytogenes* strains. The antilisterial activity of different aqueous leaf extracts against the three RTE borne *L. monocytogenes* strains is shown in Table 4. African pear leaf extract exhibited antilisterial activity against *L. monocytogenes* LMSN70 with inhibitory zones of 25 mm ± 14.14, 40 mm ±0.00, 48 mm±0.00, 50 mm± 7.00, 55 mm± 4.23, and 63 mm± 0.00 at 100 mg/mL, 200 mg/mL, 400 mg/mL, 600 mg/mL, 800 mg/mL and 1000 mg/mL respectively. Ginger leaves did not exhibit any antilisterial activities at concentration lower than 600mg/mL but displayed inhibitory zones of 17 mm ± 0.00, 25 mm ± 4.23 mm and 20 mm± 7.23 at 1000mg/mL for *L. monocytogenes* LMSN70, LMEW94, LMMP104 respectively. Bitter leaf extract displayed antilisterial activity against *L. monocytogenes* LMEW94 with zones of 35mm ± 0.00, 50mm ± 0.00, 60mm ±2.33, 64mm ± 2.23, 70mm ± 0.00, and 65mm ± 0.00 at 100 mg/mL, 200 mg/mL, 400 mg/mL, 600 mg/mL, 800 mg/mL and 1000 mg/mL respectively. Moringa and lime aqueous leaf extracts did not exhibit any antilisterial activity.

Herbal medicines are valuable for primary health care and complementary health care system. Unfortunately many plant species, which possess significant concentrations of phytochemicals of medicinal value, are yet to be discovered. Although, large numbers of plants are constantly being screened for their antimicrobial effects, the main factors that determine the antimicrobial activity are the type and composition of the plant extract, amount used, type of microorganism, pH and temperature of the environment [17]. Although several reports had been published that describe the antibacterial properties of different herbs and spices [17] only a few has focused on antilisterial activity. The antilisterial activity observed in this study could be due to the presence of some of the secondary metabolites like, tannins, saponins, terpenes and flavonoids which were detected and have previously been reported to have antibacterial activity. Comparatively, it would seem that the bioactive antilisterial compounds were more soluble in ethanolic solvent as the *F. elastica* ethanol leaf extract displayed the highest antilisterial potential at the least concentration (Table 3). Although the aqueous leaf extracts displayed lower antilisterial activities, *V. amygdalina* aqueous leaf extract exhibited maximal antilisterial activities comparable with antilisterial inhibitory growth zones elicited by the respective ethanolic extracts with the exception of *M. oleifera*leaf extracts. These trends are novel and would suggest that the ethanolic and aqueous leaf extracts of *F. elastica* and *V. amygdalina* are viable sources of bioactive antilisterial compounds. Omoya and Akharaiyi, [18] reported inhibitory growth zones of ethanol and methanol extracts of *Z. officinale* against different bacteria of public health importance. *P. gujava* leafextract was reported to have *invitro* activity against *L.*

DOI: 10.5281/zenodo.3469444

monocytogenes with 70% aqueous extract of *P. guajava* leaf having 25mm*;* methanol extract of *P. guajava* leaf had 30mm*;* ethylacetateextract of *P. guajava leaf* had 27mm and hexane extract of *P. guajava* leaf *had no* in-vitro effect [19]. *Dacryodes edulis* has been reported to have antibacterial activity against coliforms, *Escherichia coli, Salmonella typhi, Staphyloccocus aureus* and *Streptoccocusfaecalis* with zone of inhibition of 30mm, 35mm, 32mm, 34mm and 35mm respectively [20].

Conclusion

The examined strains of *Listeria monocytogenes* exhibited varying *in vitro* susceptibility patterns to the varying concentrations of ethanol and aqueous plant leaf extracts. Further studies aimed at fractionation of the respective extracts especially *F. elastica*and exposing the respective multi antibiotic resistant food borne *L. monocytogenes* strains to these fractionated leaf extracts should be conducted. Also appropriate *in vivo* antilisterial evaluation of these plant extracts using relevant mammalian models is also recommended.

Abstract

PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTILISTERIAL ATTRIBUTES OF DIFFERENT AQUEOUS AND ETHANOLIC LEAF EXTRACTS

Daniel O. Ebakota, Onilude A. Abiodun, Obayagbona Omoregbe Nosa

Introduction. *Listeria monocytogenes* represents the *Listeria* species most commonly associated with disease in humans. The majority (99%) of the infections caused by *L. monocytogenes* are food-borne being ingestion of contaminated food especially contaminated ready-to-eat food products that do not undergo subsequent reheating. This organism has great economical implications in the food industry due to recalls of contaminated food products and temporary shutdown of many food processing plants. There has been lots of interest recently in the role of complementary and alternative medicines for the treatment of various acute and chronic diseases. The revival of interest in the use of African medicinal plants by many developing countries and the World Health Organization (WHO) has led to intensified efforts to explore the numerous plants with medicinal importance. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms *in vitro*. Knowledge of the chemical constituents of plants and their anti-Listeria ability is desirable because such information will be value for synthesis of complex chemical substances. The aim of this work was to study the phytochemical qualitative profiles and *in vitro* antilisterial attributes of aqueous and ethanolic leaf extracts from several plants against earlier documented Ready To Eat (RTE) associated multi antibiotic resistant *L. monocytogenes* strains. **Materials and Methods**. The examined aqueous and ethanolic leaf extracts were derived from *Psidium guajava* (guava), *Zingiber officinale* (Ginger), *Dacryodes edulis* (African pear), *Citrus aurantifolia* (Lime), *Funtumia elastica* (silkrubber), *Vernonia amygdalina* (Bitter leaf), *Cassia alata* and *Moringa oleifera* (horseradish tree). The respective *L. monocytogenes* strains utilized were; LMSN 70, LMEW 94 and LMMP 104. *Invitro* assay of aqeous and ethanol estracts of plants was assayed by agar well diffusion assay. **Results and discussion.** Alkaloids, saponins, steroids, terpenoids, cardiac glycoside, reducing sugar, phenolics, resins, flavonoids, and tannins were detected in *Z. officinale* ethanolic leaf extract whilst cardiac glycoside was absent in ethanolic leaf extracts of *D. edulis*, *C. aurantifolia* and *F. elastica*. Alkaloids, saponins, steroids, terpenoids, cardiac glycoside, reducing sugar, phenolics, resins, flavonoids, and tannins were detected in crude aqueous leaf extracts of *Z. officinale* and *M. oleifera*.*F. elastica* ethanolic leaf extract displayed the highest antilisterial potential at the least concentration; 100mg/ml whilst amongst the aqueous extracts, *V. amygdalina* leaf extract exhibited maximal antilisterial activities comparable with antilisterial inhibitory growth zones elicited by the respective ethanolic extracts with the exception of *M. oleifera* extracts.

Conclusion. Further studies aimed at the fractionation of the respective crude extracts especially *F. elastica* and exposing the respective multi antibiotic resistant food borne *L. monocytogenes* strains to these fractionated leaf extracts should be conducted.

Keywords: Antilisterial, Agar well diffusion, Aqueous extract, Ethanolic extract, Phytochemicals, RTE borne *L. monocytogenes*

References

1. Sharma AK, Gangwar M, Tilak R [et al]. Comaparative in vitro antimalarial and phytochemical evaluation of methanolic extract of root, stem and leaf of Jatropha curcas Linn. Intern. J. Pharm. Sci. Res 2012. Vol. 3. N0 6. P. 154-1553
2. Cowan MM. Plant products as antimicrobial agents. Clin. Mic. Rev 1999. Vol. 12. No 4. P. 564-582
3. Hao YY, Brackett RE, Doyle MP. Inhibition of Listeria monocytogenes and Aeromonas hydrophila by plant extracts in refrigerated cooked beef. J. Food Pro1998. Vol 61. No.3. P 307–312.
4. Ward T J, Gorski L, Borucki MK. [et al]. Intraspecific phylogeny and lineage group identification based on the prfA virulence gene cluster of Listeria monocytogenes. J. Bac 2004. Vol 186. P 4994–5002.
5. Lis-Balchin M, Deans SG. Bioactivity of selected plant essential oils against Listeria monocytogenes. Mic1997. V 82 No 6. P. 759–762.
6. Kim JM, Marshall MR, Wei C. Antibacterial activity of some essential oil components against five foodborne pathogens. J.of Agr F. Chem1995. V 43. No11. P.2839–2845.
7. Bakr RO, Omer EA, EL-Razik KAA. [et al]. Antioxidant and anti-listerial activities of selected Egyptian medicinal plants. Afr. J. Mic. Res 2013 V 7. No.37. P 4590-4595.

DOI: 10.5281/zenodo.3469444

8. Blanco MM, Costa CARA, Freire AO. [et al]. Neurobehavioral effect of essential oil of Cymbopogon citratus in mice. Phyto. 2009. V 16. P 265–270.
9. Larson AE, Yu, RRY, Lee OA. [et al]. Antimicrobial activity of hop extracts against Listeria monocytogenes in media and in food. Intern. J. Mic1996. V 33 No. 3. P 195–207
10. Daniel OE, Onilude AA, Obayagbona O N. Prevalence of antibiotic resistant Listeria monocytogenes strains in Nigerian Ready-to eat foods. FS 2018. V. 6 No.3. P.118–125.
11. Harborne JB. Phytochemical methods. London Chapman and Hall, Ltd. 1973 P. 49-188.
12. Trease G, Evans C. A Textbook of Pharmacognosy. Twelfth edit. London: ELBS. 1985. P. 121
13. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some homostaticplants in Edo and Delta states of Nigeria. Glo. J. P. App. Sci. 2018. V. 5. P 203-208.
14. Azu N, Onyeagba R. Antimicrobial Properties of extracts of Allium cepa (Onions)and Zingiber officinale (Ginger)on Escherichia coli, Salmonella typhi and Bacillus subtilis. Intern. J.Tro.Med.2006 V. 3 No. 2 P. 1-7
15. Asowata I, Erhabor OJ, Idu M. [et al]. Preliminary antimicrobial and phytochemical study of theaqueous,alcoholic and chloroform extracts of the leaves of Napoleonaea vogelli hook. and planch. (Lecythidiaceae). J.M.B.F.S 2013 V. 2 No. 4. P. 2279-2292.
16. Adeshina GO, Jibo SD, Agu VE. Antibacterial susceptibility pattern of pathogenic bacteria isolates from vegetable salad sold in restaurants in Zaria, Nigeria. J. Mic. Res. 2012 V. 2 P. 5-11.
17. Gull IM, Saeed M, Shaukat H. [et al]. Inhibitory effect of Allium sativum and Zingiber oficinale extracts on clinically important drug resistant pathogenic bacteria. Ann.Clin. Mic. Antimic 2012 V. 11. P. 8-10.
18. Omoya FO, Akharaiyi FC. Mixture of honey and ginger extract for antibacterial assessment on some clinical isolates. Intern. Res. J. Pharma 2012 V. 2 P. 127-132.
19. Omoregie EH, Ibrahim I, Ibekwe N. [et al]. Broad spectrum antimicrobial activity of Psidium guajava linn leaf. Nat. Sci2010. V. 8. No. 12. P. 43-50
20. Nwokonkwo DC. The phytochemical study and antibacterial activities of the seed extract of Dacryodes edulis (African Native Pear). Amer. J. Sci. Ind. Res 2014 V.5. No.1. P.7-12

DOI: 10.5281/zenodo.3469444

Table 1: Phytochemical screening of various ethanolic leaf extracts

Scientific names	Common name	Alk	Sap	Ste	Tep	C. gly	R. sug	Phe	Res	Flav	Tan
Psidium guajuva	**Guava**	+	+++	+	-	+	+	+	++	+++	++
Zingiber officinale	**Ginger**	++	+++	+	++	++	+	+++	++	++	+
Dacryodes edulis	**African pear**	+++	++	++	+++	-	+	+	+	+	+
Citrus aurantifolia	**Lime**	++	+++	++	-	-	+	-	++	++	++
Funtumia elastica	**Silk rubber**	++	+++	+	+	-	+	+	+++	+	++
Vernonia amygdalina	**Bitter leaf**	++	++	+	+	++	+	++	++	+++	+++
Cassia alata	**Cassia**	++	++	++	++	+	++	+++	++	++	-
Moringa oleifera	**Moringa**	+++	+++	+++	-	++	+	++	+++	+	+

Key: Alk- Alkaloids, Sap-Saponins, Ste- Steroids, Tep- Terpenoids, C. gly: Cardiac glycoside, R. sug: Reducing sugar, Phe: Phenolics, Res: Resins, Flav- Flavonoids, Tan: Tannins, +++ = highly present, ++ = moderately present, + = slightly present, - = absent

Table 2: Phytochemical screening of different aqueous leaf extracts

Scientific names	Common name	Alk	Sap	Ste	Tep	C. gly	R. sug	Phe	Res	Flav	Tan
Psidium guajuva	**Guava**	+++	++	++	-	++	++	+++	+++	++	+
Zingiber officinale	**Ginger**	+++	++	+	+	+	++	++	+	++	++
Dacryodes edulis	**African pear**	++	+	+	+	-	++	++	+++	++	++
Citrus aurantifolia	**Lime**	++	++	+++	-	++	+	+	++	++	++
Funtumia elastica	**Silk rubber**	+	++	-	-	+	+	+	+	++	+
Vernonia amygdalina	**Bitter leaf**	++	+++	+	+	+	++	++	+	+++	+++
Cassia alata	**Cassia**	_	+	+	+	+	++	++	++	+	_
Moringa oleifera	**Moringa**	++	+	++	+	+	++	+	++	+	++

Key: Alk- Alkaloids, Sap-Saponins, Ste- Steroids, Tep- Terpenoids, C. gly: Cardiac glycoside, R. sug: Reducing sugar, Phe: Phenolics, Res: Resins, Flav- Flavonoids, Tan: Tannins, +++ = highly present, ++ = moderately present, + = slightly present, - = absent

DOI: 10.5281/zenodo.3469444

Table 3. Antilisterial activity of different ethanolic leaf extracts against food borne *L. monocytogenes* strains

	L. monocytogenes strain code	Zone of Inhibition (mm)					
Common name		**100mg/mL**	**200mg/mL**	**400mg/mL**	**600mg/mL**	**800mg/mL**	**1000mg/mL**
Guava Leaves	**LMSN70**	**30 ± 2.00**	**45 ± 14.12**	**50 ± 7.07**	**57 ± 0.00**	**60 ± 0.00**	**65 ± 0.00**
	LMEW94	**27 ± 1.25**	**38 ± 2.53**	**48 ± 5.04**	**60 ± 0.00**	**60 ± 0.00**	**65 ± 4.23**
	LMMP104	**33 ± 0.00**	**40 ± 2.14**	**47 ± 0.00**	**60 ± 0.00**	**60 ± 0.00**	**62 ± 2.28**
Ginger leaves	**LMSN70**	**0 ± 0.00**	**0 ± 0.00**	**0 ± 0.00**	**0 ± 0.00**	**30 ± 4.23**	**40 ± 0.00**
	LMEW94	**0 ± 0.00**	**0 ± 0.00**	**0 ± 0.00**	**0 ± 0.00**	**10 ± 0.00**	**25 ± 4.23**
	LMMP104	**0 ± 0.00**	**0 ± 0.00**	**0 ± 0.00**	**0 ± 0.00**	**15 ± 1.23**	**20 ± 7.23**
African pear leaves	**LMSN70**	**25 ± 14.14**	**40 ±0.00**	**40 ±0.00**	**50 ± 7.00**	**55 ± 4.23**	**40 ± 0.00**
	LMEW94	**25 ± 14.14**	**40 ±0.00**	**40 ±0.00**	**50 ± 7.20**	**55 ± 4.23**	**40 ± 0.00**
	LMMP104	**25 ± 14.14**	**40 ±0.00**	**40 ±0.00**	**50 ± 7.00**	**55 ± 0.00**	**40 ± 0.00**
Lime leaves	**LMSN70**	**20 ± 3.23**	**25 ± 7.00**	**25 ± 7.07**	**30 ± 14.14**	**35 ± 7.07**	**55 ± 5.03**
	LMEW94	**15 ± 0.00**	**20± 1.23**	**20 ± 0.00**	**20 ± 7.53**	**30 ± 4.23**	**35 ± 2.00**
	LMMP104	**20 ± 7.10**	**25 ± 7.00**	**30 ± 0.00**	**33 ± 3.13**	**35 ± 7.07**	**40 ± 5.03**
Silk rubber leaves	**LMSN70**	**55 ±7.10**	**65 ± 3.00**	**70 ±0.00**	**60 ± 2.53**	**50 ± 0.00**	**40 ±0.00**
	LMEW94	**30±0.00**	**45 ± 1.00**	**55 ±0.00**	**70 ± 0.00**	**50 ± 0.00**	**40 ±0.00**
	LMMP104	**45 ±0.00**	**55 ± 0.00**	**65 ±0.00**	**72 ± 2.33**	**70 ± 0.00**	**40 ±0.00**
Bitter leaf	**LMSN70**	**30 ± 0.00**	**50 ± 0.00**	**55 ±7.07**	**63 ± 3.45**	**70 ± 7.07**	**40 ± 0.00**
	LMEW94	**35 ± 0.00**	**50 ± 0.00**	**60 ±2.33**	**64 ± 2.23**	**70 ± 0.00**	**35 ± 0.00**
	LMMP104	**33 ± 0.00**	**50 ± 0.00**	**62 ±0.00**	**66 ± 7.03**	**70 ± 0.00**	**43 ± 0.00**
Moringa leaf	**LMSN70**	**0 ± 0.00**	**0 ± 0.00**	**0 ± 0.00**	**0 ± 0.00**	**0 ± 0.00**	**0 ± 0.00**
	LMEW94	**0 ± 0.00**	**0 ± 0.00**	**0 ± 0.00**	**0 ± 0.00**	**0 ± 0.00**	**0 ± 0.00**
	LMMP104	**0 ± 0.00**	**0 ± 0.00**	**0 ± 0.00**	**0 ± 0.00**	**0 ± 0.00**	**0 ± 0.00**
Cassia leaf	**LMSN70**	**10 ± 0.00**	**22 ± 8.43**	**25 ± 1.45**	**32 ± 0.00**	**38 ± 2.56**	**37 ± 3.04**
	LMEW94	**8 ± 0.00**	**15 ± 0.00**	**20 ± 7.12**	**25 ± 0.00**	**30 ± 0.00**	**32 ± 0.00**
	LMMP104	**7 ± 0.00**	**12 ± 2.43**	**18 ± 2.24**	**26 ± 0.00**	**34 ± 2.42**	**34 ± 2.04**

DOI: 10.5281/zenodo.3469444

Table 4: Antilisterial activity of different aqueous leaf extracts against food borne *L. monocytogenes* strains

Common names	*L. monocytogenes* strain code	Zone of Inhibition (mm)					
		100mg/mL	200mg/mL	400mg/mL	600mg/mL	800mg/mL	1000mg/mL
Guava Leaves	LMSN70	15 ± 7.07	25 ± 7.07	30 ± 0.00	33 ± 4.94	40 ± 0.00	45 ± 0.00
	LMEW94	27 ± 1.25	38 ± 2.53	48 ± 5.04	50 ± 0.00	52 ± 0.00	50 ± 4.23
	LMMP104	17 ± 0.00	23± 2.14	27 ± 0.00	36 ± 0.00	48 ± 0.00	50 ± 2.28
Ginger leaves	LMSN70	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	13 ± 4.23	17 ± 0.00
	LMEW94	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	10 ± 0.00	25 ± 4.23
	LMMP104	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	15 ± 1.23	20 ± 7.23
African pear leaves	LMSN70	25 ± 14.14	40 ±0.00	48 ±0.00	50 ± 4.23	55 ± 4.23	63 ± 0.00
	LMEW94	25 ± 14.14	40 ±0.00	40 ±0.00	50 ± 7.00	59 ± 4.23	65 ± 0.00
	LMMP104	22± 3.53	42± 4.50	65 ± 0.00	60 ± 0.00	70 ± 0.00	72 ± 3.52
Lime leaves	LMSN70	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
	LMEW94	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
	LMMP104	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Silk rubber leaves	LMSN70	10 ± 0.00	20 ± 0.00	30 ± 0.00	40 ± 14.12	50 ± 7.07	55 ± 14.14
	LMEW94	15±0.00	25 ± 1.00	28±0.00	40 ± 0.00	50 ± 0.00	40 ±0.00
	LMMP104	17 ±0.00	19 ± 0.00	25 ±0.00	42 ± 2.33	54 ± 0.00	53 ±0.00
Bitter leaf	LMSN70	30 ± 0.00	50 ± 0.00	55 ±7.07	63 ± 3.45	70 ± 7.07	50 ± 0.00
	LMEW94	35 ± 0.00	50 ± 0.00	60 ±2.33	64 ± 2.23	70 ± 0.00	65 ± 0.00
	LMMP104	33 ± 0.00	54 ± 0.00	62 ±0.00	66 ± 7.03	69 ± 0.00	73 ± 0.00
Moringa leaf	LMSN70	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
	LMEW94	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
	LMMP104	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Cassia leaf	LMSN70	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
	LMEW94	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
	LMMP104	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00

DOI: 10.5281/zenodo.3469444