Journal of Advances in Biology & Biotechnology
6(1): 1-6, 2016; Article no.JABB.26026
ISSN: 2394-1081

SCIENCEDOMAIN *international*
www.sciencedomain.org

Inhibition of Major *Listeria monocytogenes* Serotypes Grown in Media Supplemented with Aqueous Extracts of *Gongronema latifolium* and *Vernonia amygdalina*

Ogueri Nwaiwu[1,2*]

[1]*Division of Food Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, LE12 5RD, College Road, Loughborough, Leicestershire, United Kingdom.*
[2#]*Research Services Division, Alpha-Altis (UK) Ltd, Sir Colin Campbell Building, University of Nottingham Innovation Park, Triumph Road; Nottingham, NG7 2TU, United Kingdom.*

Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/JABB/2016/26026
Editor(s):
(1) Anil Kumar, Professor & Head (Chair), School of Biotechnology, Devi Ahilya University, Madhya Pradesh, India.
Reviewers:
(1) M. D. Salihu, Usmanu Danfodiyo University, Sokoto, Nigeria.
(2) Hatice Taner Saraçoğlu, Selcuk University, Turkey.
Complete Peer review History: http://sciencedomain.org/review-history/14417

Short Communication

Received 30th March 2016
Accepted 25th April 2016
Published 2nd May 2016

ABSTRACT

Aims: To determine the growth inhibitory effect of extracts of *Gongronema latifolium* and *Vernonia amygdalina* on major *Listeria monocytogenes* serotypes.
Study Design: Preliminary analytic observational studies.
Place and Duration of Study: University of Nottingham, United Kingdom; Study was carried out between September 2012 and September 2013.
Methodology: The major serotypes of *Listeria monocytogenes* serotypes 1/2a, 1/2b, 4b and 1/2c found in evolutionary lineage I and II were grown in media containing aqueous extract of *Gongronema latifolium* and *Vernonia amygdalina* obtained from a 250 g/l leaves decoction. The percentage growth inhibition relative to the control was determined by taking viable counts of *L. monocytogenes* after growth for 24 hours at 30°C in Brain Heart Infusion broth supplemented with 10, 20, and 30% w/v of the extracts.

**Corresponding author: E-mail: ogueri.nwaiwu@alpha-altis.co.uk;*
 # Present Address.

Results: It was found that there was no significant growth reduction for media supplemented with 10 and 20% (v/v) of the extracts. However significant ($p < 0.5$) inhibition was observed for cultures of *L. monocytogenes* serotype 4b grown in media supplemented with 30% (v/v) of extracts of *V. amygdalina*. The highest growth reduction was 29% and overall, more inhibition was found with extracts of *V. amygdalina* than extracts of *G. latifolium*.
Conclusion: Aqueous extracts of *G. latifolium* and *V. amygdalina* appeared to inhibit major serotypes of *L. monocytogenes* but they were not bactericidal. Serotypes 4b maybe more sensitive to the extracts than other serotypes. If further purification of the aqueous extracts studied is carried out, microbial retardants could be developed which may help to delay the growth of *L. monocytogenes* where ever they are prevalent.

Keywords: Viability; inhibition; Gongronema latifolium; Vernonia amygdalina; leaf extracts; Listeria monocytogenes.

1. INTRODUCTION

Extracts from plant material especially from leaves in the form of herbs and spices have been used since ancient times because of their antimicrobial properties which enable them to act against foodborne pathogens and spoilage bacteria [1]. An important food-borne pathogen transmitted to humans via contaminated food is *Listeria monocytogenes* [2] which causes listeriosis. Those that are especially high risk patients are the young, elderly, immunosuppressed persons and pregnant women [3]. The bacterium has the ability to cause serious invasive disease in ruminants and humans and is widely distributed in the environment. When listeriosis occurs, it can be difficult to control and usually results in severe clinical outcomes [4]. The organism has a 20% to 30% case fatality rate and a small fraction of strains (serotypes 1/2a, 1/2b, and 4b) is usually associated in human listeriosis [5].

Around the world, especially in Asia, Africa and Latin America, people use plants extracts for treatment of diseases. The effect of plant extracts on food borne pathogens has been studied by many workers and the methods used to quantify inhibition may include minimum inhibitory concentration (MIC) and zone of inhibition after a disc diffusion assay [6], or growth reduction relative to a control after viable count [7]. It has been documented that factors affecting inhibition by any antimicrobial substance can be biological or technical. Biological factors include persistence, paradoxical effect, tolerance and phenotypic resistance whereas technical factors can be growth phase of inoculum, inoculum size, insufficient contact, volume transferred and choice of media [8].

In Nigeria leaves of two plants botanically known as *Gongronema latifolium* and *Vernonia amygdalina* or locally known in south eastern Nigeria as *Utazi* and *Onugbu* respectively are very important and are widely used. Leaves of *G. latifolium*, a rain forest plant is used as a spice and vegetable and well linked to many medicinal properties [9]. Also the leaves of *V. amygdalina* are used for bitter leaf soup and also have medicinal properties [10]. It is not uncommon to find people in the rural areas preparing herbal mixtures with the leaves of these two plants for the treatment of several ailments. In the urban areas, mixtures containing these two herbs are sold in unlabeled containers and the sellers tend to attract a lot of patrons who believe in the efficacy of the herbal mixtures. The herbs have not been commercialized possibly because a holistic trial with animals and humans confirming the adequate dose and active compounds required to bring health benefits and disease reversal have not been carried out.

The medicinal use of extracts of leaves from *G. latifolium and V. amygdalina* is well reported in literature, but there are few reports on the effects of the aqueous extracts on viability of the major serotypes of *L. monocytogenes*. Therefore, the aim of the study was to investigate if the viability of *L. monocytogenes* serotypes is inhibited in the presence of the aqueous extracts of the two plants under study.

2. MATERIALS AND METHODS

2.1 Samples and Extract Preparation

Samples of sundried *G. latifolium and V. amygdalina* (Fig. 1) were purchased in Owerri, South Eastern Nigeria and sent to the University of Nottingham, United Kingdom where the work was carried out. Aqueous extracts were prepared

as previously described [11]. Briefly, a decoction from chopped sun dried leaves was prepared by adding 1000 ml of distilled water to 250 g of *G. latifolium* or *V. amygdalina* leaves and boiled in a stainless steel pot until approximately 50% of original volume was remaining. Further processing was carried out by straining the mixture into Duran bottles after which the mixture was autoclaved at 121°C for 15 min and then allowed to stand and cool. The mixture was transferred into 30 ml centrifuge tubes and centrifuged (MSE, UK) at 3000 x g for 5 min after which the supernatant was filtered through a membrane of 0.45 µm (Millipore) pore size and stored at 4°C for use. Up to 30 ml per extract was frozen at -20°C for future investigations.

2.2 Reconstitution of *L. monocytogenes* Strains

Strains of major *L. monocytogenes* serotypes of evolutionary Lineages I and II in the culture collection of University of Nottingham, United Kingdom (Table 1) were reconstituted from -85°C by streaking cryo preservation beads onto Brain Heart Infusion (BHI) agar (Oxoid) and grown at 30°C for 48 h. Colonies that emerged were sub-cultured on BHI plates and grown again at 30°C for 48 h.

2.3 Assessment of Viable Growth Inhibition by Leaf Extracts

A loopful from a colony of reconstituted cells for each strain studied was used to inoculate 10 ml of BHI broth after which it was incubated at 30°C for 18 h. To establish if extracts can show inhibitory activity, a 10, 20, and 30% (w/v) mixture of the extracts of *G. latifolium* or *V. amygdalina* and 1 ml of the 18 h culture was made up to 100 ml with BHI broth in a 250 ml conical flask and grown with shaking for 24 h after which 100 µl of the mixture was spread-plated on Tryptone Soya agar plates [6]. The control consisted of 1 ml of the 18 h culture made up to 100 ml without any extracts. The percentage bacterial count of cells that emerged on the Tryptone Soya plates after 48 h incubation relative to control [7] was used to quantify inhibition. Three independent sets of mixtures consisting of culture, extract and broth of *G. latifolium* or *V. amygdalina* were assessed.

2.4 Statistical Analysis

Analysis of variance and Student's T tests were carried out with the Data Analysis ToolPak of Microsoft© Excel 2010.

3. RESULTS AND DISCUSSION

3.1 Effects of *V. amygdalina* Extracts

Efforts to integrate data on *L. monocytogenes* persistence are still needed for a better understanding of the prevalence and persistence of the organism [14]. This study explores the potential of inhibiting *L. monocytogenes* growth with plant extracts. The growth percentage relative to control for cells grown in 10 or 20% (v/v) of *V. amygdalina* showed an inhibition of less than 10% and was not significant ($p > 0.05$; Fig. 2a) However significant differences ($p<0.05$) were observed for cells grown in media supplemented with up to 30% (v/v) of prepared extracts which showed up to 29% reduction. The *L. monocytogenes* serotype 4b implicated in many outbreaks of listeriosis and represented by the reference strain ATCC 23074 in this study was the most sensitive to *V. amygdalina* extracts (Fig. 2a). It is possible that the sensitivity observed was because *L. monocytogenes* serotype 4b is well known to be more prevalent in clinical samples than environmental samples and normally thrives in human or animal hosts rather than the environment. Overall, the

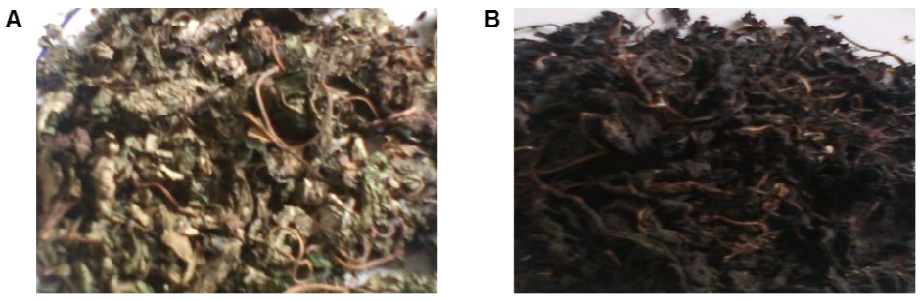

Fig. 1. Samples of sundried chopped leaves of *G. latifolium* (A) and *V. amygdalina* (B)

Table 1. Test strains used in this study

Strain	Serotype	Evolutionary lineage	Source	Reference
Lm 4	1/2b	I	Milk	[12]
Lm 27	1/2c	II	Food environment	[12]
Lm 10403S	1/2a	II	Clinical	[13]
Lm ATCC 23074	4b	I	Clinical	ATCC

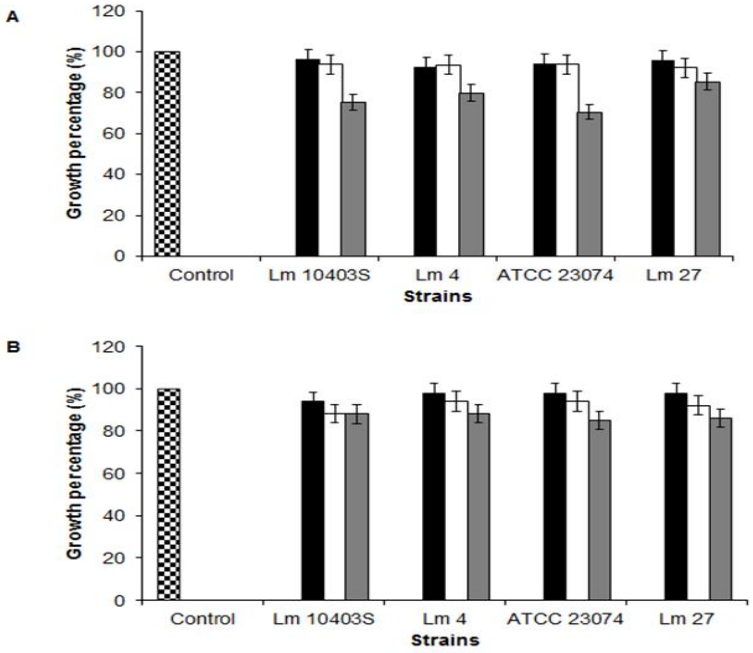

Fig. 2. Growth percentage relative to the control (▩) of major molecular serotypes of *L. monocytogens* grown in 10% (■), 20% (□), and 30% (▣) aqueous extracts of *V. amygdalina* (panel A) and *G. latifolium* (panel B) prepared from leaves decoction. Extracts were used as supplements in BHI medium and mixture was grown at 30°C for 24 h after which viable count was carried out

inhibitions seen with 10 and 20% (v/v) of the extracts and the highest reduction of up to 29% (Fig. 2a) observed for serotype 4b is far from the 99.99% bactericidal action required for a compound to be classified as an antimicrobial agent [15]. However combining these extracts with other antimicrobials on further purification may have the benefit of being a food grade microbial retardant. The extracts of *V. amygdalina* have shown good synergy when combined with doxorubicin and it was suggested that it can complement current chemotherapy in the treatment of cancer [16].

In Nigeria, folklore medicine attributes the efficacy of *V. amygdalina* to the famous bitter taste of the leaves. The bitterness varies and some varieties of the plant are not very bitter. The specific compounds reported to be responsible for the bitter taste among others are four specific known sesquiterpene lactones which includes vernodalin, vernolide, hydroxyvernolide and vernodalol [17]. These bitter compounds among other derivatives have been tested [18] for antibacterial activity against Gram-positive bacteria but the test did not include *L. monocytogenes.* In the study, strong inhibitions were found for *Bacillus subtilis* and *Micrococcus lutea* but no link was made between degree of bitterness and antimicrobial action. Another study [19] carried out following extraction of vernolide and vernodalol showed that both compounds exhibited a significant bactericidal activity against five Gram positive bacteria while lacking efficacy against the Gram negative strains. Also, vernolides exhibited high activity against *Penicillium notatum*, *Aspergillus flavus, Aspergillus niger* whereas vernodalol

showed moderate inhibitions against *Aspergillus flavus*, *Penicillium notatum* and *Aspergillus niger*.

3.2 Effects of *G. latifolium* Extracts

The reduction in growth for cells growing in media supplemented with *G. latifolium* was observed but it was not significant ($p > 0.05$) and was not up to the reduction observed for cells grown in media supplemented with *V. amygdalina* (Fig. 2b). The highest reduction of up to 15% was observed for the same strain ATCC 23074 (serotype 4b) that showed the highest growth reduction for cells grown in media supplemented with *V. amygdalina*. The inhibition of *L. monocytogenes* by *G. latifolium* has been demonstrated. An investigation [20] identified the constituents of *G. latifolium* and later tested aqueous and methanol extracts against pathogenic bacterial isolates and it was found that the aqueous extracts showed no activity against *Enterobacter faecalis, Yersinia enterolytica* among others. The aqueous extracts did not show any activity on *L. monocytogenes* for the range tested. However, the study showed that methanol extracts were active against *Pseudomonas aeruginosa* and *L. monocytogenes* but the particular serotype of *L. monocytogenes* that showed this sensitivity was not described.

4. CONCLUSION

Aqueous extracts of *G. latifolium* and *V. amygdalina* appears to inhibit major serotypes of *L. monocytogenes* but they are not bactericidal. More inhibition is seen with extracts of *V. amygdalina* than extracts of *G. latifolium* and the *L. monocytogenes* serotype 4b showed more sensitivity than other major serotypes. It would be beneficial to sample a wider variety of both plants taking into consideration the biological and technical factors especially the age of the plant after harvest and extract active ingredients to determine the conditions that can cause the strongest inhibition.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Nabavi SF, Di Lorenzo A, Izadi M, Sobarzo-Sánchez E, Daglia M, Nabavi SM. Antibacterial effects of cinnamon: from farm to food, cosmetic and pharmaceutical industries. Nutrients. 2015;7(9):7729-48.
2. Reda WW, Abdel-Moein K, Hegazi A, Mohamed Y, Abdel-Razik K. *Listeria monocytogenes*: An emerging food-borne pathogen and its public health implications. J Infect Dev Ctries. 2016;10(2):149-54.
3. Hernandez-Milian A, Payeras-Cifre A. What is new in listeriosis? Biomed Res Int; 2014.
(Accessed 31 October 2015)
Available:http://dx.doi.org/10.1155/2014/358051
4. Maertens de Noordhout C, Devleesschauwer B, Angulo FJ, Verbeke G, Haagsma J, Kirk M, et al. The global burden of listeriosis: A systematic review and meta-analysis. Lancet Infect Dis. 2014;14(11):1073-82.
5. Nyarko EB, Donnelly CW. *Listeria monocytogenes*: Strain heterogeneity, methods, and challenges of subtyping. J Food Sci. 2015;80(12):M2868-78.
6. Alcaráz LE, Fusco MR, Mattana CM, Satorres SE, Laciar AL. Antibacterial activity of methanolic and aqueous extracts of *Ligaria cuneifolia* and *Tripodhantus flagellaris*. Emir J Food Agric. 2015; 27(11):831-36.
7. Birdi T, Daswani P, Brijesh S, Tetali P, Natu A, Antia N. Newer insights into the mechanism of action of *Psidium guajava* L. leaves in infectious diarrhea. BMC Complement Altern Med. 2010;10.
(Accessed January 2 2016)
Available:http://www.biomedcentral.com/1472-6882/10/33
8. Clinical and Laboratory Standards Institute (CLSI). Methods for determining bactericidal activity of antimicrobial agents. Approved Guideline, CLSI Document M26-A, CLSI, 950 West Valley Roadn Suite 2500,Wayne, Pennsylvania 19087, USA; 1999.
(Accessed 15 June 2013)
Available:http://shop.clsi.org/site/Sample_pdf/M26A_sample.pdf
9. Ugochukwu NH, Babady NE. Antioxidant effects of *Gongronema latifolium* in hepatocytes of rat models of non-insulin dependent diabetes mellitus. Fitoterapia. 2002;73(7-8):612-18.
10. Farombi O, Owoeye O. Antioxidative and chemopreventive properties of *Vernonia amygdalina* and *Garcinia biflavonoid*. Int J Environ Res. Public Health. 2011;8(6): 2533-55.

11. N'guessan K, Amoikon EK, Tiébré M, Kadja B, Zirihi GN. Effect of aqueous extract of *Chrysophyllum cainito* leaves on the glycaemia of diabetic rabbits. Afr J Pharm Pharmacol. 2009;3(10):501-6.
12. Lawrence LH, Hill HA, Linton M. DNA sequence divergence within the *iap* gene of *Listeria monocytogenes* as a means of characterizing strains. In: XII International symposium on Problems of listeriosis; 1995. Perth, Australia.
13. Bishop DK, Hinrichs DJ. Adoptive transfer of immunity to *Listeria monocytogenes*: The influence of *in vitro* stimulation on lymphocyte subset requirements. J Immunol. 1987;139(6):2005-9.
14. Ferreira V, Wiedmann M, Teixeira P, Stasiewicz MJ. *Listeria monocytogenes* persistence in food-associated environments: Epidemiology, strain characteristics, and implications for public health. J Food Prot. 2014;77(1):150-70.
15. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. J Pharm Anal; 2015.
    DOI: 10.1016/j.jpha.2015.11.005.
16. Wong FC, Woo CC, Hsu A, Tan BKH. The anti-cancer activities of *Vernonia amygdalina* extract in human breast cancer cell lines are mediated through caspase-dependent and p53-independent pathways. Plos One. 2013;8(10):e78021. DOI: 10.1371/journal.pone.0078021
17. Ohigashi H, Jisaka M, Takagaki T, Koshimizu K, Huffman MA, Nishida T, et al. Possible medicinal plants used by wild chimpanzees, and their physiologically active compounds. J. Afr Stud. 1991;39: 15-27.
18. Jisaka M, Ohigashi H, Takegawa K, Huffman MA, Koshimizu K. Antitumoral and antimicrobial activities of bitter sesquiterpene lactones of *Vernonia amygdalina*, a possible medicinal plant used by wild chimpanzees. Biosci Biotech Biochem. 1993;57(5):833-4.
19. Erasto P, Grierson DS, Afolayan AJ. Bioactive sesquiterpene lactones from the leaves of *Vernonia amygdalina.* J Ethnopharmacol. 2006;106(1):117-20.
20. Eleyinmi AF. Chemical composition and antibacterial activity of *Gongronema latifolium*. J Zhejiang Univ Sci B. 2007; 8(5):352-8.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14417