Antibacterial Susceptibilty of PhilNONI Juice versus *Salmonella typhi, Staphylococcus aureus,* and *Escherichia coli*

Florita S. Maslog,
QC/QA and Production Manager, Phil Morinda Citrifolia, Inc. (PMCI)
Los Banos, Laguna, Philippines

Abstract

Juice extracted from noni fruit (*Morinda citrifolia, Linn*) is a natural dietary supplement that has been claimed to benefit human health. Limited clinical tests from medical doctors and food scientists all over the world have testified to its beneficial effects. The secret in health giving power lies in the number and variety of components found in the noni fruit. A scientific approach to understanding the phenomenon why and how it has become a popular dietary supplement, as a functional food, or as a natural health enhancer is due to the fact that *Morinda citrifolia* contains phytochemicals that own antibacterial, antiviral, antifungal, antitumor, anti-helmintic, analgesic, hypotensive, anti-inflammatory, and immune- enhancing effect (Maslog, 2014). This in-vitro study focuses on the inhibitory effect of M. citrifolia juice using the branded product,100% PhilNONI Juice, of a local company, Phil Morinda Citrifolia Inc. (PMCI), versus pathogenic microorganisms *Salmonella typhi, Staphylococcus aureus* and *Escherichia coli*. Two methods were used in determining the antimicrobial susceptibility of PhilNONI Juice: one is to find out the minimum inhibitory concentration (MIC) by the tube dilution method and the second is the determination of the juice's antibacterial activity against these pathogenic organisms assessed by the presence or absence of growth by the streak method with the pathogenic organisms by Todd, Sanford, Davidsohn (Henry, 1979). The study proved that 100% PhilNONI Juice has a potent antibacterial activity against *Salmonella typhi, Staphylococci aureus* and *Escherichia coli* based on the same pattern of antimicrobial susceptibility of standard chloramphenicol on these pathogenic organisms.

Keywords: susceptibility, inhibition of growth, antibacterial

Introduction

The increase in antibiotic resistance of microorganisms is largely due to the widespread use of antibiotics in medicine (Rivera et al., 2011). The problem is compounded by the lack of new antibiotics to attack bacteria in different ways to circumvent the resistant ones. Moreover, commercially available antibiotics are sometimes associated with adverse reactions to the hosts such as hypersensitivity, allergic reactions, and immunosuppression. These adverse effects led us to search for new antibacterial substances from local fruits. Antimicrobials of organic plant origin can have enormous therapeutic potentials. Looking for more effective antimicrobial agents from organically grown fruits, such as noni fruit, is the purpose of this in-vitro study.

This study is also inspired by Republic Act no. 8423, an act creating the Philippine Institute of Traditional and Alternative Health Care (PITAHC), otherwise known as the "Traditional and Alternative Medicine Act (TAMA) of 1997. This Act has its main objectives to encourage scientific research to develop traditional and alternative health care systems that have direct impact on public health care and promote and advocate the use of traditional, alternative, preventive and curative health care modalities that have proven safe, effective, affordable, and consistent with the Philippine government standards on medical practice. These very well conform with the mission and objectives of Phil Morinda Citrifolia, Inc. (PMCI), the manufacturer of PhilNONI products. For ages, nature has gifted us with plenty of herbs and plants which form the main source of traditional medicine. A study on the antibacterial activities of ethanol extracts of some of these medicinal plants in the Philippines against multidrug resistance bacteria had been done in 2015 (Valle et al., 2015).

Morinda citrifolia, Linn (Rubiaceae), also known as noni orapatot is a shrub or a small tree growing organically and wildly along the seashores of the Pacific Ocean. This shrub has been used widely in traditional medicine but has been used more recently as analgesics, anti-angiogenic, anti-cardiovascular diseases,

anti-inflammatory, anti-oxidants, anti-tumor/cancer, anti-gastrointestinal diseases, as well as an antimicrobial and as an immunomodulatory for immune responses (Maslog, 2014).

Specific compounds from the noni plant have been effective in laboratory studies as antibacterial agents (Lochner et al., 1995; Leach et al., 1998). In an in- vitro study, extracts of ripe noni fruit containing L-asperuloside alizarin exhibited antibacterial properties against *Pseudomonas aeruginosa*, *Micrococcus pyogenes, E. coli, Proteus spp., Staphylococcoccus aureus, Bacillus subtilis, Salmonella* , and *Shigella*. Some anthraquinones from the root, the damnacanthal, is also found effective against all the above organisms. Specifically, scopoletin component in noni fruit inhibits *E. coli and H. pylori* (Duncan, 1998). Also concentrated noni leaf extract kills 89% of *M. tuberculosis* in test tubes, compared to Rifampin, a leading antiTB drug giving 97% effectivity in the same concentration (Saludes, 2002).

Chloramphenicol, also known as chloromycetin, is used as the standard control antibiotic in this study. It is a broad spectrum antibiotic that interferes with the mitochondrial protein synthesis and is active against a variety of organisms including Salmonella and in the treatment of penicillin–allergic or penicillin-resistant patients with bacterial meningitis and infections caused by vancomycin-resistant *Enterococci*. Therapeutic range in adults is 5-20 ug/ml. (Bambecke, 2017). Chloramphenicol is also bacteriostatic for most gram-positive, like Staphylococcus aureus and many gram-negative aerobic bacteria, like *Escherichia coli,* but can be bactericidal against some very sensitive bacteria. All anaerobic bacteria are inhibited by chloramphenicol at its usual therapeutic concentrations. Chloramphenicol suppresses growth of *Rickettsia* and *Chlamydia.* Its pharmacokinetic action is well-distributed throughout the body, including CNS and the eyes.

Materials and Methods

Two Methods for Antibiotic Sensitivity Testing by Todd, and Sanford, Davidsohn (Henry, 1979) were used in this study.

1. Test tube Dilution Method. Tubes of nutrient vehicle containing serial dilutions of antibiotic are inoculated with known number of test organism. The measure of susceptibility to the antibiotic is manifested by failure of organism to grow in a given dilution. The lowest dilution without growth is the minimum inhibition concentration (MIC). This is the most accurate method. The minimum bactericidal concentration (MBC) is a confirmatory test.
2. Streak Method. Mueller Hinton agar (MHA) plates containing known amounts of antibiotic are streaked with the different number of test organism and reading in which volume and antibiotic concentration there is inhibition of growth is the MIC.

Sources of the Microorganisms Used

The microorganisms used in this study were purchased from the Philippine National Collection of Microorganism, (PNCM) at the National Institutes of Molecular Biology and Biotechnology (BIOTECH), in the University of the Philippines Los Banos (UPLB), under PNCM locator no. 18012301. These microorganisms were Salmonella typhi, BIOTECH 1756 ; *Staphylococcus aureus* , BIOTECH, 1582 and *Escherichia coli* ,BIOTECH 1634. The three (3) bacterial cultures from the NA slant were gram stained to confirm their gram stain reactions and streaked on differential media for their specific cultural and biochemical characteristics.

Methodology I

1a. Determination of the Minimum Inhibitory Concentration (MIC) by Test Tube Dilution Test

Table 1

Chloramphenicol Antibiotic (AB) Diluted to Working Concentration of 250 ug/ml Serially Diluted 1:2 in 5 mL Volume and Challenged with 1 x 10^8 Organisms: Salmonella Typhi (A) Staphylococcus Aureus (B) and Escherichia coli (C) except Tube no. 9 with Nutrient Broth Alone

Test Tube No	Dilution	AB in ug/ml	Results (Remarks)
1	undiluted	250.00	I,(S)
2	1:2	125.00	I,(S)
3	1:4	62.50	I,(S)
4	1:8	31.25	I,(S)
5	1:16	15.62	I,(S)
6	1:32	7.81	S (MIC)
7	1:64	3.90	R
8	NB+org	0	G
9	NB alone	0	NG

Legend: I= Inhibition of growth, S= Sensitive R= Resistance, G=growth NG=No growth

Table 2

100%PhilNONI Juice Diluted 1:32 and Using 1 ml Equivalent to MIC of 7.81 ug/ml and Challenged with 1 x 10^8 Test organisms: Salmonella Typhi (A) Staphylococcus aureus (B) and Escherichia coli (C)

100% PhilNONI Juice Diluted 1:32 1 ml =7.81 ug/ml compared to Chlorampenicol	MIC Results from the Chloramphenicol run	Average of 8 replicates	Positive Control No PhilNONI juice,	Negative Control Media alone, No challenge
vs. A	7.81 ug/ml	I	G	NG
vs. B	7.81 ug/ml	I	G	NG
vs. C	7.81 ug/ml	I	G	NG

Legend: I= Inhibition of growth G= growth NG= no growth
MIC=Minimum inhibition concentration

1b. Determination of Minimum Bactericidal Concentration (MBC), Confirmatory to Minimum Inhibitory Concentration (MIC)

Table 3

Culture on MHA of three (3) random results showing MICs done on Chloramphenicol (AB) from table 1 with 1 x 10^8 test organism after one day incubation at 37°C. Determination of Minimum Bactericidal Concentration (MBC)- Confirmatory test for the MIC

MIC of 7.81 ug/ml Chloramphenicol	REPLICATES			Pos. Co. 3.90 ug/ml
	1	2	3	
with *Salmonella typhi* (A)	I	I	I	G
with *Staphylococcocus aureus* (B)	I	I	I	G
with *Escherichia coli* (C)	I	I	I	G

Table 4

Culture on MHA of Three (3) Random MICs Done on 100% PhilNONI Juice from Table 2 with 1 x 10^8 Test Organism, after Incubation for a Day At 37°C. Minimum Bactericidal Concentration (MBC)

MIC of 7.81 ug/ml PhilNONI Juice	REPLICATES			Pos. Co. 3.90 ug/ml
	1	2	3	
with *Salmonella typhi* (A)	I	I	I	G
with *Staphylococcocus aureus* (B)	I	I	I	G
with *Escherichia coli* (C)	I	I	I	G

Legend: I = Inhibition of growth G= growth

Methodology II Determination of Growth Inhibition at Certain Antibiotic Concentration by Streak Method

Table 5

Chloramphenicol (AB) in different volumes and concentration plated on MHA and streaked with 1 x10^8 organisms : Salmonella typhi (A), Staphylococcus aureus (B) and Escherichia coli (C)

Petridish No.	Vol. AB mL	Conc. AB mcg/ mL	Results for 8 Replicates for each of A,B & C organisms								Remarks
			1	2	3	4	5	6	7	8	
1	1.0	7.81	I	I	I	I	I	I	I	I	S(MIC)
2	1.5	11.71	I	I	I	I	I	I	I	I	S
3	2.0	15.62	I	I	I	I	I	I	I	I	S
4	2.5	19.52	I	I	I	I	I	I	I	I	S
5	0	0	G	G	G	G	G	G	G	G	+Co.

Table 6

100% PhilNONI Juice 7.81 ug/l in different volumes and concentration plated on MHA and streaked separately with 1 x 10^8 Salmonella typi (A) and Staphylococcus aureus (B) except no. 5

Petridish No.	Vol. of 100% PhilNONIin ug/ml	Results for 8 Replicates for each of A and B								Remarks
		1	2	3	4	5	6	7	8	
1	1.0ml=7.81	I	I	I	I	I	I	I	I	S(MIC)
2	1.5ml=11.71	I	I	I	I	I	I	I	I	S
3	2.0ml=15.62	I	I	I	I	I	I	I	I	S
4	2.5ml=19.52	I	I	I	I	I	I	I	I	S
5	0	G	G	G	G	G	G	G	G	+Co.

Table 6a

PhilNONI Juice 7.81 ug/ml in Different Volumes and Concentration Plated on MHA and Streaked with 1 x 10^8 Escherichia Coli

Petridish No.	Vol. AB mL	Replicates for C								Remarks
		1	2	3	4	5	6	7	8	
1	1.0ml=7.81	G	G	G	G	G	G	G	G	R
2	1.5ml=11.71	I	I	I	I	I	I	I	I	S(MIC)
3	2.0ml=15.62	I	I	I	I	I	I	I	I	S
4	2.5ml=19.52	I	I	I	I	I	I	I	I	S
5	0	G	G	G	G	G	G	G	G	+Co.

Results and Discussion

Antimicrobial sensitivity or antimicrobial susceptibility is the susceptibility of microorganism, usually bacteria, to antibiotics. Testing for antibacterial susceptibility is often done clinically by Kirby–Bauer method, (Henry, 1979) but this method is qualitative and not very reliable for our purpose. Two other methods employed in this study were those of the broth and agar dilution methods for minimum inhibitory concentration (MIC) determination which are both quantitative and more accurate.

PhilNONI Juice exhibited exactly the same pattern of reactions shown by chloramphenicol by the test tube dilution method. Results shown in Table 2 of the in- vitro antibacterial susceptibility test was only the MIC of 100% PhilNONI Juice which was 7.81 ug/ml using the constant volume of approximately 1 x 10^8/ml test organisms S*almonella typhi, Staphylococcus aureus*, and *Escherichia coli* inhibiting the growth of the three organisms. This is even a better result than the study of Thamyris et al. (2014) in Brazil, which reported that ethanolic extract of M. citrifolia fruit had antibacterial activity inhibiting *Salmonella spp, Staphylococcus aureus* and *Eschrichia coli* with MIC ranging from 1mg/ml to 10 mg/ml. Perhaps the advantage of the present study is in the method of the noni fruit preparation. Thamyris and co-workers (2014) used the extracted oil while in the present study the whole fermented juice was used, making it more potent with more components preserved and used in the reactions.

The present study collaborates with other studies already reported regarding the potent antimicrobial activity noni may exhibit. Jian Yang et al. (2016) showed an average of 0.3 ul/cm2 MIC of noni fruit essential oil for both *E. coli* 0157:H7 and *Salmonella enteritidis*. In still another study by Indian workers (Barani, et al., 2014), the MIC values of noni extracts for *Streptococci mutans* and *Streptrococci mitis* were found to be 125ug/ml and 62.5 ug/ml respectively, using 1 x 10^7 count of the organisms from dental caries.

The factors present in noni that inhibit microbial growth may be attributed not only to the ethanol formation from natural fruit fermentation but also to the presence of phenolic compounds in the fruit like the anthraquinones, acubin, asperuloside, alizarin and scopoletin. Around 160 phytochemical had been isolated from *M. citrifolia* including the above phenolic compounds, organic acids, and alkaloids that contributed to its action of inhibiting pathogenic bacteria growth, including *Staphylococcus aureus* and *Escherichia coli* (Barani et al., 2014).Yang et al. (2016) attributed this antimicrobial characteristic of noni fruit to caprylic, acid which they isolated through the GC-MS analysis in 2014, with MIC values against *E. coli* O157:H7 and *S. enteritidis* of 3.6 and 4.33 ul/ml, respectively.

Moreover, in the presence of the helpful Lactobacilli or lactic acid bacteria (LAB), which are resident in noni fruits, another reaction called lactic acid fermentation happens in processing the PMCI 's 100% PhilNONI Juice, illustrated as follows (Arioli et al., 2013).

$C_6H_{12}O_6 \rightarrow 2\ CH_3CHOHCOOH + C_2H_5OH + CO_2$
Glucose lactic acid ethanol

In this stage, decarboxylation of L-malic to lactic acid by the action of LAB increases the acidity of the juice. This is also the stage where the juice is less susceptible to any further damage from other bacteria since LAB has used up all the substrate and has secreted bacteriocin or biocide. This antibiotic-like substance kills pathogenic bacteria and prevents growth of other microorganisms. The biocide's mode of action is to block the lipid synthesis in *E.coli* and other microbes (Arioli et al., 2013). The conversion of malic acid

to lactic acid is the height of juice fermentation that imparts flavor and aroma to the juice, which gave LAB the status of "Generally Recognized as Safe" (GRAS) through the American Food and Drug Agency (Amorr et al., 2007). As confirmatory test, Table 4 shows the almost complete inhibitory action as can be observed from the replicated MICs formed by 100% PhilNONI Juice, as compared to the standard chloramphenicol antibiotic action which indicated that 100% PhilNONI Juice eliminated the three test bacteria. In contrast, 3.90 ug/ml showed some growth on MHA in all random samples, serving as control. Table 6 and Figure 1 show that when different volumes of 100% PhilNONI Juice were plated in MHA and streaked with 1 x 10^8 test organisms, the juice behaved in the same pattern as the standard chloramphenicol antibiotic. *Salmonella typhi* and *Staphylococcus aureus* growth was inhibited by 7.81 to 19.5 ug/ml of the antibacterial substance in noni fruit, based on the exact action of chloramphenicol of the same concentrations, as shown in Table 5. *E. coli*, however, must contain 11.7-19.5 ug/ml biocide-like substance to eliminate 1 x 10^8 count of the test organisms, as can be observed in Table 6a.

The results confirm Todd, Sanford, and Davidsohn's recommendation to use MIC for chloramphenicol of 5-15 ug/ml in order for it to be considered sensitive. One hundred percent PhilNONI Juice antibacterial activity falls almost within the MIC recommendation by Todd, Sanford, Davidsohn (Henry, 1979). The results indicate that the juice has a potent antibacterial activity against *Salmonella typhi, Staphylococcus aureus* and *Escherichia coli.*

Figure 1

Determination of MIC of Chloramphenicol each vs. the 3 Organisms Salmonella typhi (A), Staphylococcus aureus (B) and Escherichia coli (C)

Neg.co,NB& Noni Juice 250ug/ml 125ug/ml 62ug/ml 31ug/ml 15.6ug/ml 7.81ugml(MIC)

NG NG NG NG NG NG NG NG

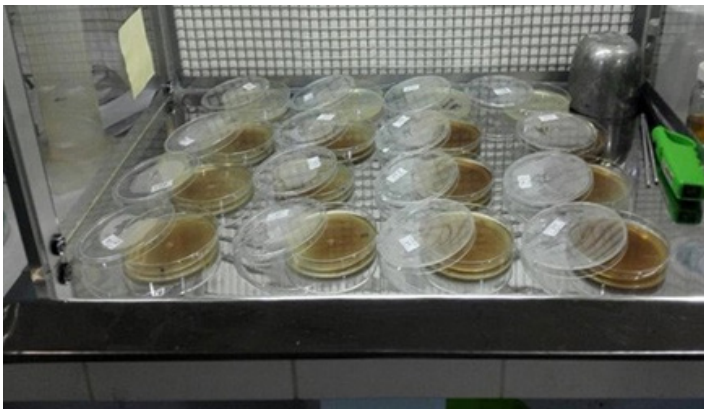
Figure 2

Test Tube Dilution Method: Tubes Containing Serial Dilution of 100% PhilNONI Juice to Check its Antibiotic Susceptibility to Constant Amount of Pathogenic Organism. The same results were seen with Salmonella typhi (A), Staphylococcus aureus (B) and Escherichia coli (C).

ug/ml	250	125	62.5	31.2	15.6	7.81	3.9	Neg co.(outside rack)
No growth		NG	NG	NG	NG	NG	G	No growth

Figure 3

Streak Method: Different amounts of 100% PhilNONI Juice and Chloramphenicol Poured and Solidified on MHA, then Streaked with the 1 x 10^8 Test Organisms. All Plates Showed no Growth



Conclusion and Recommendation

One hundred percent PhilNONI Juice has a potent antibacterial activity as shown by the test tube dilution and streak methods of Todd, Sanford, Davidsoh. The MIC result was based on exactly the same pattern as antimicrobial susceptibility of chloramphenicol antibiotic against *Salmonella typhi, Staphylococcus aureus* and *Escherichia coli*, the three pathogenic bacteria Food and Drug Administration (FDA) are concerned about in food businesses. This study indicates that noni juice not only serves as health food supplement giving many benefits but can also give protection from bacterial infections when taken. In view of the growing resistance to antibiotics, any variants of PhilNONI product may have the potentials to be used as an antibiotic against salmonellosis, food toxicity, and other skin infections and gastroenteritis.

References

Amorr, M.S., Florez, A.B., & Mayo, B. (2007). Antibiotic resistance in non-enterocallactic acid bacteria and bifidobacteria. *Food Microbiol*, 24, 559-570.

Arioli, S. , Elli, M., Ricci, G., & Mora, D. (2013). Assessment of the susceptibility of lactic acid bacteria to biocides. *Int. J of Food Microbiol,* 163, 1-5.

Aryal S. (2015). Mueller Hinton Agar. Composition, principle , uses and preparation microbiology info .*Online Microbiology Notes* .

Bambeke, F. V . (2017). *Chloramphenicol, in infectious diseases* (4th ed.). ASM Press.

Barani, K. , Sunayana, M. Prabu, D., Adil, A. Mohanaganesh S., & Jeevika, C. (2014). Role of aqueous extract of *Morinda citrifolia* (Indian noni) ripe fruits in inhibiting dental caries –causing *Streptococcus mutans and Streptococcus mitis. Int. J ClinPediatr Dent, 11*(6), 703-710.

Duncan , S., Flint, H.J., & Stewart, C.S.(1998). Inhibitory activity of gut bacteria against *Escherichia coli* 0157H:7mediated by dietary plant metabolites. *Fems Microbiol Lett, 164,* 283-285.

Henry, J. B. (1979). *Todd, Sanford, Davidsohn Clinical diagnosis and management by laboratory methods.* London.

Jian, Y. Afaisen, S. J., & Gadi, R. (2016). Antimicrobial activity of noni fruit essential oil on *Escherichia coli* 0157:H7 and S*almonella enteritidis. Micronesica,* 05, 1-10.

Leach, A. J. , Leach D.N., & Leach, G.J. (1998). Antibacterial activity of some medicinal plants of Papua New Guinea. Sci. New Guinea, 14, 1-7.

Lochner C.P. , Burch, M. , Mower, H. , Berestecky, J. , Davis, H., van Poel, B., Lasure, A., Vanden, D., & Vlietinck, A. (1995). Antimicrobial activity and anti-complement activity of extracts obtained from selected Hawaiian medicinal plants. *J Ethnopharmacol, 49*(1), 23-32.

Maslog, F . (2014). The pharmacological values, medicinal uses and mode of action of the different known components of noni fruit (*Morindacitrifolia)*: A review of literature. Prepared for Phil MorindaCitrifolia, Inc. (PMCI).

Republic Act no. 8423. 1997. Traditional and Alternative Medicine Act (TAMA).

Rivera, A., Giono, S., Gonzalez, M., Rodriguez, N., & Cedillo, L. (2011). Antibacterial effect of *Morinda citrifolia* fruit juice against *Mycoplasma. Annals of Biol. Res, 2*(3), 491-497.

Saludes,J. P. et al. (2002). Anti-tubercular constituents from hexane fraction of *Morinda citrifolia Linn,* (Rubiaceae). *Phytother Res., 16*(7), 683-685.

Thamyris, C. , de Franca, J.P., Chaves, A.L.F., & Lopes F.A.R. (2014). Evaluation of antitumoral and antimicrobial activity of *Morinda citrifolia L.* grown in Southeast Brazil. *Acta Cirurgica Brasileira, 29*(2), 10-14.

Valle, D., Andrada, J., Puzon, J., Cabrera, E., & Rivera, W. (2015). Antibacterial activities of ethanol extracts of Philippine medicinal plants against multi-drug resistant bacteria. *Asian Pacific J of Trop Bio Med., 5*(7), 532-540.