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Antibacterial Activity of *Morinda citrifolia* Fruit Juice

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ABSTRACT: *Morinda citrifolia* (Noni) is widely used in traditional Asian medicine for decades. Noni fruit juice has been reported for its various functional effects including antibacterial activity. However, previous reports was mainly emphasized on fresh noni juice whereas the effect of fermented juice has not yet well established. Uncontrolled fermentation conditions such as natural microbial and fermentation time especially in house and small factory production are of important for health benefit and safety of consumers. The objective of this study was to evaluate antibacterial effect of 7 noni juices that produced by different processes namely fresh noni juice (FJ), 1 year fermented by pure and natural microbe with sugar (FP 12 and FN 12), 6 months fermented by pure and natural microbe with sugar (FPS 6 and FNS 6), and 6 months fermented by pure and natural microbe without sugar (FPNS 6 and FNNS 6). Five bacterial were employed: *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus feacalis*, *Escherichia coli* and *Klebsiella pneumoniae*. Antibacterial ability was assessed by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). MIC concentrations of FJ, FP 12, FN 12, FPS 6, and FNS 6 were in a range of 31.25-62.5 µl/ml. Moreover, it was found that MBC of FJ was as high as 250-500 µl/ml which implies that FJ can only inhibit bacteria by bacteriostatic effect of active compound, not killing. Whereas MBC of fermented noni juice by natural and pure microbial with sugar (FP 12, FN 12, FPS 6 and FNS 6) were 31.25-62.5 µl/ml, similar to their MIC values. This infers that these noni juices have bactericidal activity. On the other hand, when fermented noni juices produced by either natural or pure microbial but without sugar (FPNS 6 and FNNS 6) were considered, MIC and MBC concentrations were similarly high at 250 µl/ml. Though, their high MIC concentration showed low antibacterial activity but killing effect also observed. This is the first report of antibacterial activity of different processes of noni juice. It can be concluded that two fermentation time ranges (6 or 12 months) had no effect on antibacterial activity of noni juice. In addition, pure and natural microbe fermenter also did not alter those activities. Nevertheless, sugar added fermentation was the main factor that increased antibacterial activity of noni juice.

Keywords: Noni, *Morinda citrifolia*, Antibacterial activity, MIC, MBC

INTRODUCTION

Morinda citrifolia is known commonly as Noni. It is planted widely throughout the Pacific. It has been used in folk medicine by Polynesians for over 2000 years. It is reported to have a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, antihelmin, analgesic, hypotensive, anti-inflammatory, and immune enhancing effects [1].

Alternative medicine benefits from many parts of noni plant were approved including fruit [2], Leaf [3], seed [4] and root [5]. Ripen noni is beneficially used for noni juice production. The fresh juice from ripen fruit has been confirmed for its antibacterial activities as a result of bioactive substances such as phenolic compounds and, in case of fermented juice, acid which is a product from lactic acid fermentation process [6]. Noni juice from ripen noni fruit was mostly used in many reports for antibacterial effect demonstration [2].

In Thailand, commercial noni juices are widely produced in house or small factory. There are 2 production types of noni juice; pasteurized and fermented noni juice. Until now, there is no study of the change in active compounds occurring from different process. Moreover, quality control of fermented noni juice is not achievable when natural bacteria which consist of unknown types and number are employed. For these reasons, producers, government organizations, private agencies as well as customers are questioning for the safety of commercial fermented noni juice hence market possibility of noni juice is limited.

Objective of this study was to evaluate physicochemical properties, bioactive compounds and antibacterial effect of noni juice that produced from different producing processes. Factors affecting fermentation process were varied including fermentation time, sugar content and microbial type (natural or pure culture).

MATERIAL AND METHODS

1 Noni juice

The 7 noni juices were produced by different processes namely pasteurized fresh noni juice (FJ), 1 year fermented by pure and natural microbe with sugar (FP 12 and FN 12), 6 months fermented by pure and natural microbe with sugar (FPS 6 and FNS 6), and 6 months fermented by pure and natural microbe without sugar (FPNS 6 and FNNS 6). The brief processes are described below.

1.1 Pasteurized fresh noni juice

Fully ripe noni fruits were cleaned, chopped and thoroughly blended. Noni puree was filtered and the juice was collected. Then the juice was pasteurized at 80 ^{o}C for 1 min prior to storage at –20 ^{o}C until use.

1.2 Fermented noni juice by pure microbe

Fully ripe noni fruit was cleaned and chopped. To the fruits, 40% w/w of 50% sugar syrup was added. Temperature of the mixture was then brought up to 70 ^{o}C for 10 min. Pasteurized noni mixture was ice-shocked in a closed fermentation tank. Ten percentage of pure microbial stock was added after cooling down prior to fermentation at room temperature for 6 or 12 months. During fermentation, fermented gas was regularly released. Fermented noni was added with 15 % pasteurized water before juice extraction. Clear fermented noni juice was kept at –20 ^{o}C for further analysis. For non-sugar added formula, similar method was employed except for excluding the addition of sugar syrup.

The pure microbial stock was freshly prepared. The mixture of chopped ripe noni fruit (70 % w/w), brown sugar (10% w/w) and water (20% w/w) was heated at 70 ^{o}C for 10 min before cooling with ice-cold bath. Ten percentage of pure culture, *Lactobacillus casei* (TISTR

1340), was added into the mixture prior to fermentation for 24 hrs.

1.3 Fermented noni juice by natural microbe
Ingredients and processing of fermented noni juice by natural microbe (with or without sugar) was similar to that of fermented noni juice by pure microbe, except for the mixture was not pasteurized and pure microbial stock was not utilized.

2 Chemical property determination

2.1 Total phenolic content
The total phenolic content in each sample was determined by spectrophotometer according to Folin-Ciocalteu procedure [7]. Total phenolic content of sample was estimated from a calibration curve of gallic acid at 760 nm. The total phenolic content of sample was expressed as mg gallic acid equivalent (GAE) per mL of sample.

2.2 Total Flavonoid content
Total flavonoid content determination was carried out according to the aluminium chloride colorimetric assay [8]. 1 mL of noni sample or standard solutions of catechin was added to 4 mL of distilled water. Then 0.30 mL 5% NaNO2 was added followed by 0.3 mL 10 % AlCl3 after 5 min. Five minutes after, 2 mL IM NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance at 510 nm was read against the blank. The total flavonoid content was expressed as mg catechin equivalents (CE).

2.3 Ferric reducing antioxidant power (FRAP) assay

FRAP assay was modified from the method of Benzie and Strain [9]. An aliquot of 200 µL noni samples were mixed with 3 mL FRAP reagent in test tubes. The mixture was incubated in water bath for 30 minutes at 37 ^{o}C and the absorbance of the samples was determined against blank at 593 nm. Standard curve was prepared at a range of stock solution concentrations at 200, 400, 800, 1200 and 1600 µM using aqueous solution of $FeSO_4.7H_2O$. FRAP value was expressed as µmole Ferrous sulfate/mL sample.

2.4 DPPH Assay
Evaluation of radical DPPH scavenging activity of noni samples was carried out as described previously [10]. To 3.8 mL ethanol solution of 0.1 mM DPPH radical, 0.2 mL of sample was added. The mixture was well mixed and left to stand at room temperature in the dark for 30 min. The absorbance at 517 nm was measured against ethanol blank. The percent of DPPH discolouration of the sample was calculated. DPPH value was expressed as trolox equivalent/ mL sample using the calibration curve of Trolox. Linearity range of the calibration curve was 20 to 1000 µM.

2.5 Scopoletin and damnacanthal
Scopoletin and damnacanthal were analysed by HPLC-DAD/MSD. The reference methods were verified and carried out by The Central Laboratory (Thailand) Co. Ltd., Chiang Mai, Thailand.

3 Test bacteria

Five standard bacteria *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6635), *Enterococcus feacalis* (ATCC 51299), *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 700603) were cultivated on trypticase soy agar (TSA) plates. For working cultures, 3-4 colonies were suspended in trypticase soy broth (TSB), incubated for 1 hr, at 37°C. Then the culture was diluted to provide a final inoculum of approximately 10^6 CFU/ml

4 Determination of MIC and MBC Values

Broth microdilution assays were performed according to standard method M07-A9 of Clinical and Laboratory Standards Institute (CLSI) with modifications [11]. Briefly, 20 µL

of 10^6 CFU/mL of bacteria was inoculated into 180 µL of noni juice which serial 2 fold diluted in TSB. Prior to aerobic incubation at 37°C for 24 hrs, minimum inhibitory concentration (MIC) values were determined by observing the turbidity. The lowest concentrations of noni juice with clear suspensions were considered as the MIC values. The lowest concentrations of noni juice in post-incubation suspensions which did not harbour any bacterial growth upon culturing on TSA after overnight incubation at 37 °C were considered as the Minimum Bactericidal Concentration (MBC) values.

RESULT AND DISCUSSION

Chemical property of noni juice products

Table 1 illustrates total phenolic and flavonoid content and antioxidant capacities of seven noni samples produced with different conditions: pasteurized noni juice (FJ), 1 year fermentation employing pure and natural microbe in the presence of sugar (FP 12 and FN 12), 6 months fermentation employing pure and natural microbe in the presence of sugar (FPS 6 and FNS 6), and 6 months fermentation employing pure and natural microbe without sugar (FPNS 6 and FNNS 6).

Among different recipes, FPS 6 had the highest total phenolic content. Type of fermentation microbe (pure and natural) did not lead to a significant difference of phenolic content. It is also obvious that fermentation time had no major effect on total phenolic content but the presence of sugar is of most important. As can be seen, both FPS 6 and FNS 6 gave rise to a marked increase of total phenolic content whereas the lowest values were evident in non-sugar fermented juices (FPNS 6 and FNNS 6). Surprisingly, Fresh juice contained low phenolic content in a similar level with FPNS 6 and FNNS 6. Preliminary study showed that phenolic content of fresh noni juice increased during fermentation process. This was thought to be due to plant cell wall breakdown by microorganisms hence releasing of bioactive compounds was encouraged. The comparable effects were also seen in flavonoid content FRAP and DPPH values (Table 1).

Scopoletin and damnacanthal are major bioactive compounds that responsible for health benefits of noni. In the current study, it was found that the highest bioactive compounds, both scopoletin and damnacanthal, were noted in fresh juice (FJ) and FPS 6. Scopoletin content in FJ and FPS 6 were 54.49 and 53.41 mg/kg, respectively. Likewise, damnacanthal content in the two samples were 67.20 and 68.17 mg/kg, respectively. It is crucial to point out that high amount of bioactive compounds were found in fresh noni juice and the fermentation process and sugar content had no major effect.

MIC and MBC of noni products

Seven noni juices were determined for MIC by broth microdilution method, following with MBC, against five bacteria. Bacteria employed were both gram positive and negative bacteria (Tables 3 and 4). The MIC and MBC results against Gram negative (GN) bacteria show in Tables 5 and 6 and Figure 2.

Table 1 chemical properties of noni juice products

Noni juice	Total phenolic content (mgGAE/ 100 mL)	Flavonoid content (µg CE/mL)	FRAP (µmole Ferrous sulfate/mL)	DPPH (µmole Trolox/mL)
FJ	79.7 ±21.7^{c}	5.2±0.5^{c}	112.4 ± 25.9^{cd}	72.7 ± 0.2^{d}
FJ 12	133.3 ± 11.7^{b}	22.5±1.3^{a}	146.4 ± 12.9 ^{bc}	73.5 ± 0.0^{abc}
FN 12	133.4 ± 9.9^{b}	21.3±1.9^{a}	125.5 ± 10.5^{c}	73.8 ± 0.1^{a}
FPS 6	174.3 ± 19.4^{a}	21.3±2.3^{a}	193.1 ± 17.6^{a}	73.6 ± 0.1^{abc}
FPNS 6	78.7 ± 7.4^{c}	14.3±2.6^{b}	81.6 ± 15.^{1d}	73.3 ± 0.3^{c}
FNS 6	155.1 ± 8.7^{ab}	22.7±1.3^{a}	165.0 ± 11.9^{ab}	73.7 ± 0.1^{ab}
FNNS 6	94.2 ± 19.^{3c}	8.2±1.2^{c}	119.5 ± 25.4^{c}	73.4 ± 0.2^{bc}

^{abc}Mean values in the same column bearing different superscripts are significantly different ($p < 0.05$).

Table 2 Scopoletin and damnacanthal content in selected noni juice products

Noni juice	Scopoletin (mg/kg)	Damnacanthal (mg/kg)
FJ	54.49	67.20
FPS 6	53.41	68.17
FPNS 6	44.31	48.11
FNS 6	44.67	61.42
FNNS 6	38.82	55.32

Table 3 MIC of noni juice against Gram positive bacteria

Noni juices	Concentration (µL/mL)		
	S. aureus	*B. subtilis*	*E. faecalis*
FJ	31.25	62.50	31.25
FP 12	31.25	31.25	31.25
FN 12	31.25	31.25	31.25
FPS 6	31.25	31.25	31.25
FPNS 6	250	250	250
FNS 6	31.25	31.25	31.25
FNNS 6	250	250	250

Table 4 MIC of noni juice against Gram negative bacteria

Noni juices	Concentration (µL/mL)	
	E. coli	*K. pneumoniae*
FJ	31.25	62.50
FP 12	31.25	31.25
FN 12	62.50	62.50
FPS 6	62.50	31.25
FPNS 6	250	250
FNS 6	31.25	31.25
FNNS 6	250	250

Table 5 MBC of noni juice against Gram positive bacteria

Noni juices	Concentration (µL/mL)		
	S. aureus	*B. subtilis*	*E. faecalis*
FJ	250	250	250
FP 12	31.25	31.25	62.50
FN 12	31.25	31.25	31.25
FPS 6	31.25	31.25	62.50
FPNS 6	250	250	250
FNS 6	31.25	31.25	31.25
FNNS 6	250	250	250

Table 6 MBC of noni juice against Gram negative bacteria

Noni juices	Concentration (µL/mL)	
	E. coli	*K. pneumoniae*
FJ	500	500
FP 12	31.25	62.50
FN 12	62.50	62.50
FPS 6	62.50	62.50
FPNS 6	250	250
FNS 6	62.50	62.50
FNNS 6	250	250

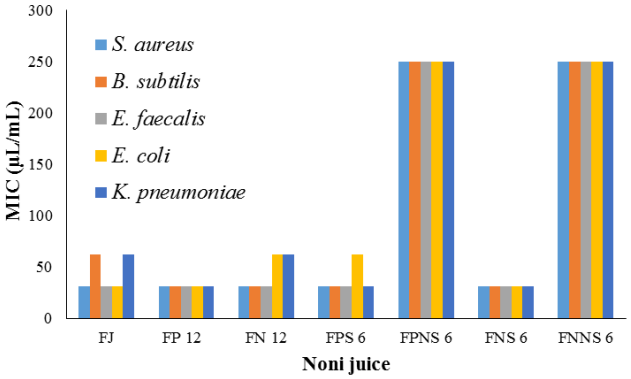

Figure 1 Minimum inhibitory concentration (MIC) of different noni juices against five bacteria

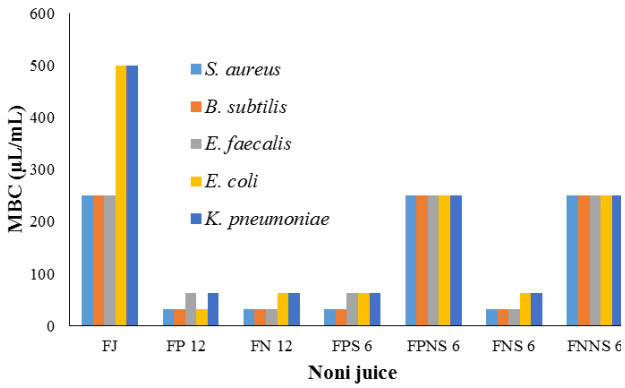

Figure 2 Minimum bactericidal concentration (MBC) of different noni juices against five bacteria

MIC of noni juices against both GP and GN bacteria ranged from 31.25-62.5 µL/mL, except for non-sugar adding fermented juices, FPNS 6 and FNNS 6, where MIC was as high as 250 µL/mL. The MIC results indicated that sugar is the key ingredient in fermentation process which increased the antibacterial activity of noni juices. Moreover, according to the chemical property of noni samples (Table 1), total phenolic content may, partly, respond to these effects. Surprisingly, MIC of fresh juice against five bacteria were also in the low ranges. This may infer that scopoletin and damnacanthal are important keys as described previously by Chan-Blanco and colleagues [12]. In current study, the high content of these bioactive compounds were also demonstrated in fresh juice compare to other samples (Table 3).

The following experiment was carried out to study MBC of noni juices against both GP and GN bacteria (Tables 5 and 6 and Figure 2). MBC of all samples were either the same or higher than MIC. In case of FJ, MBC against GN and GP were 250 and 500 µl/ml, respectively. These results indicated that antibacterial activity of active compound in FJ is bacteriostatic whereas bactericidal activity was illustrated in other noni juices, same MIC and MBC values. Likewise, although FPNS 6 and FNNS 6 showed high concentration of MIC at 250 µl/ml, but MBC was similar. The results obtained from this study are consistent with the previous reports where lactic acid fermentation of herbal plants enhanced various bio-active properties [13]. Furthermore, the different fermentation time, 6 months and 1 year, did not influence the difference antibacterial activity, thus the less time is enough for effective fermented juice preparation.

Noni juices that fermented by pure lactic acid bacteria (FP12, FPS6 and FPNS6) did not demonstrate different antibacterial activity compared to noni juices that fermented by natural microbial (FN12, FNS6 and FNN6). It can be said that natural and pure fermentation process similarly produce important antimicrobial compounds, as evident in Tables 1 and 2. Nevertheless, pure lactic acid starter culture is recommended in noni fermentation process, especially in industrial scale, because quality and safety are in concerned.

Moreover, It is not surprising that MIC of noni juices against GN was higher than GP. This indicated that GN bacteria are more tolerant to active ingredient in noni juices than GP. This can be explained by the complexity of outer compartment of GN bacteria such as cell membrane. The membrane of which has more selective permeability than GP bacteria [5].

CONCLUSIONS

Antibacterial compound in fresh noni juice is bacteriostatic, not bactericidal. Fermentation process in noni juice enhances bactericidal activity, probably because of bactericidal compound production. Sugar is a key ingredient in fermentation that promotes the antibacterial activity of fermented noni juice. Fermentation by natural microbe produce antibacterial compound in the similar manner as pure lactic acid bacteria employment. Moreover, the duration of fermentation 6 months is enough of producing the antibacterial effective compound of noni juice.

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