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Antioxidant Activity and Chemical Constituents Identification by LC-MS/MS in Bio-fermented Fruit Drink of *Morinda citrifolia* L.

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Abstract

Noni bio-fermented drink is a fermented fruit of *Morinda citrifolia* L. Noni fruit is reported to be useful for a wide range of maladies, and consumers throughout the world perceive similar benefits. However, the existing evidence does have some limitations as far as its general application to noni fruit products. This study is intended to assess the quality of noni bio-fermented drink. The fruit was fermented using wild environmental yeast by a local entrepreneur. After 3 months, the mixture was filtered, sweetened, pasteurized, and bottled. The finish product was investigated for its antioxidant activity, total phenolic content and phytochemical constituents. Antioxidant activities exhibited profound results in term of 2,2-diphenyl-1-picryhydrazyl (DPPH) assay with an IC_{50} value of 14.94±0.76 μg/mL which was very close to that of Trolox at 8.47±0.16 μg/mL. The low phenolic content of 0.75±0.01 mg gallic acid equivalent (GAE)/g measured by Folin-Ciocalteu reagent method. The data of mass spectra and their fragmentations from LC-MS/MS identified 53 of 59 phytochemical compounds from the drink. Iridoid glucosides; asperulosidic acid, deacetylasperulosidic acid and monotropein as well as coumarin; aesculetin, scopoletin were identified as markers of this drink. Moreover, amino acids including organic acids, sugars and sugar in glycosidic forms were elucidated. Sulfur compounds in this drink namely 2-Sulfanylpropan-1-ol; 3-sulfanylpropan-1-ol, thiodiglycol and L-Methionine were identified as the possible cause of the pungent characteristic fragrance. As a result, noni fruit bio fermented drink encompasses numerous nutrients and biological compounds with potent antioxidant activity which could be stated as wellness drink for health merit.

Keywords: *Morinda citrifolia* L., Antioxidant activity, Chemical constituents, LC-MS/MS, Bio-fermented drink

Introduction

Noni (*Morinda citrifolia* L.) is classified in Rubiaceae family. The tree grows well in the tropical area such as South pacific, Polynesian Island, Southeast Asia and Australia. In Thailand, it was called "Yo-baan". Mostly young leaves and fruits are used for consumption as vegetables while the other parts used for medicinal purposes. Young leaves were favorable for cooking in raw or cooked form in many dishes including fish curry. The ripe fruits possess a strong pungent smell with bitter taste and have numerous seeds. Therefore, the ripe fruits were preferably transformed into fermented juice by adding sugar and starter culture.

In Tahiti, noni fruit juice was one of the first whole foods approved under the European Union's 1997 novel food regulations [1]. The Chinese government has also approved one source of noni juice as a new resource and has approved it as a functional food that can enhance immunity [2]. People in other regions prepare their Noni fruit juice and claim it as dietary supplement for healing many ailments. Lee and his colleague [3] isolated 5 compounds from Hawaiian noni fruit juice namely: asperulosidic acid, rutin, nonioside A, (2E,4E,7Z)-deca-2,4,7-trienoate-2-O-β-d-glucopyranosyl-β-d-glucopyranoside, and tricetin. These compounds exhibited potential anti-inflammatory activity in lipopolysaccharide (LPS)-stimulated

RAW 264.7 cells. They inhibited the production of nitric oxide (NO), and down regulated the expression of IKKα/β, I-κBα, and NF-κB p65. Furthermore, treatment with these 5 compounds downregulated the expression of nitric oxide synthase and cyclooxygenase-2 (COX2). Another 2 new iridoid glucosides, 6α-hydroxyadoxoside and 6β,7β-epoxy-8-epi-splendoside and 17 known compounds, americanin A, narcissoside, asperuloside, asperulosidic acid, borreriagenin, citrifolinin B epimer a, citrifolinin B epimer b, cytidine, deacetylasperuloside, dehydromethoxygaertneroside, epi-dihydrocornin, D-glucose, D-mannitol, methyl α-D-fructofuranoside, methyl β-D-fructofuranoside, nicotifloroside, and β-sitosterol 3-O-β-D-glucopyranoside were found in noni fruit from Ohio state, USA. Americanin A showed a potent antioxidant in both DPPH and peroxynitrite ($ONOO^-$) scavenging activity test [4]. For more research data Liu and co-workers found 2 novel glycosides, 6-O-(beta-D-glucopyranosyl)-1-O-octanoyl-beta-D-glucopyranose and asperulosidic acid, extracted from the juice of noni fruits inhibit AP-1 transactivation and cell transformation in the mouse epidermal JB6 cell line [5]. Furthermore, the analysis of 7 noni fruits and 13 commercial fruit juices originating from the Caribbean, Central America, the Central and South Pacific, and Asia revealed that scopoletin, rutin, quercetin, and 5,15-dimethylmorindol were detected in all the samples, although, at varying concentrations. These compounds were suggested to be used as references for identification and authentication of raw noni fruits and their commercial products [6]. More research in Hawaiian fermented noni juice exudates, Youn and Chang [7] reported 5 compounds, heptanyl 2-O-β-D-xylofuranosyl-(1→6)-β-D-glucopyranoside (1), n-butyl β-D-glucopyranoside (2), (1S)-(3-ethenyl-phenyl)-1,2-ethanediol (3), (2S)-2-hydroxybutanedioic acid (4), and daucosterol (5). The isolated compounds were evaluated for their cancer chemo-preventive potential based on their ability to inhibit nitric oxide (NO) production and tumor necrosis factor alpha (TNF-α)-induced NF-κB activity, and quinonone reductase-1 (QR1)-inducing effect. Among the isolates, n-butyl β-D-glucopyranoside, (1S)-(3-ethenyl-phenyl)-1,2-ethanediol showed moderate quinone reductase-1 (QR-1) inducing activities, whereas all of them showed weak or no inhibitory activities against the TNF-α-induced NF-κB and NO production. Moreover, the phytochemical biological activity, pharmacological and clinical trials health effect have been extensively summarized [8-10]. Although, there have been several studies with various noni fruit and extracts. The phytochemical compositions of these extracts are considerably different than that of noni fruit juice. Therefore, the conclusions drawn from these studies may not be applicable to noni bio-fermented juice.

In year 2020 U2T (University to Tambon (meaning sub-district)) program encouraged researchers from nearly university work on field survey and help the local artisan in OTOP (One Tambon One Product) by applying scientific theories to the product-making process. Coordinating the packaging and distribution of finished products. Using scientific knowledge to blend different juice varieties together to make a new product. Planning for bottling of drink once it has matured and make sure that quality is evaluated and maintained when the noni drink is bottled. Each bottle of noni drink will highlight the story of the small local producer as an entrepreneur.

Geographical factors and variations in processing methods are known to produce commercial noni juice products with diverse phytochemical and nutrient compositions. Therefore, other sources of noni products may have different pharmacological profiles. This current research work will provide information of the phytonutrient and biological activities of noni bio-fermented drink from the Tup Yai Chiang sub-district, Phrom-phiram district Phitsanulok leading to exploration of its potential utilization as a valuable ingredient for the local functional food aiming for industrial application.

Materials and methods

Chemical and Reagents

All chemicals used were of analytical grade and obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (LC-MS grade) was purchased from RCI Labscan, (Bangkok, Thailand). Formic acid (AR grade) was obtained from Merck (Darmstadt, Germany). Purified water was purified by a Milli-Q purification system from Millipore (Bedford, MA, USA).

Sample Preparation

Noni fruits (*Morinda citrifolia* L.) in mature stage was harvested from a Tup Yai Chiang area in Phitsanulok, Thailand. The fruit was cleaned and kept in plastic bag to ensure completely ripened state. The steps in producing the fermented noni fruit juice including: (1) ripened fruits cut into pieces; (2) 3 kg of cut fruits weighed; (3) cut fruits placed into plastic containers; (4) 1 kg of sugar added; (5) all components mixed well; (6) covered and sealed in plastic containers left to ferment for 3 months with occasional stirring; (7) 2 L of water added to the fermented mixture before filtered to separate sludge from the juice; (8) the

fermented and filtered fruit juice were adjusted to taste, pasteurized and placed in bottles. During the fermentation process, pH and %brix of the fermented mixture was monitored.

Determination of Total Phenolic Content (TPC)

The total phenolic content was performed using the Folin-Ciocalteu assay with 96-well microplates [11]. Firstly, 25 µL of sample was mixed with 25 µL of 3-fold-diluted Folin-Ciocalteu reagent and 200 µl of water. After 5 min, 25 µL of sodium carbonate solution (10.6 g/100 mL) was added to the mixture and the well plate was shaken for 20 s. The mixture was incubated at room temperature in the dark for 60 min and the absorbance was measured at 725 nm by a microplate reader (BioTek Instruments, Winooski, Vermont, USA). Total phenolic content was expressed as mg of gallic acid equivalents per gram of sample. All measurements were done in triplicate.

DPPH free radical scavenging assay

DPPH assay was selected as a method for testing antioxidant activity, which reported previously [12], with some modifications. Briefly, a 150 µL of 0.2 mM DPPH was mixed with 75 µL of sample, and a control solution containing all reagents was prepared without the sample. The mixture was incubated in the dark at room temperature for 30 min and was then measured at 515 nm by a microplate reader (BioTek Instruments, Winooski, USA). Trolox was used as the positive control. DPPH radical scavenging activity was calculated using the equation: Free radical scavenging (%) = $[(A_{control} - A_{sample})/A_{control}]\times100$ where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the sample, respectively. The IC_{50} value was obtained by nonlinear regression analysis of dose-response curve plotting between %free radical scavenging and various concentrations of samples using GraphPad Prism 5.

LC-ESI-QTOF-MS/MS analysis

Liquid sample was filtered through a 0.22 um Nylon membrane syringe filter before submitted to the system. Chromatographic experiments were performed with an Agilent 6540 Q-TOF-MS spectrometer (Agilent Technologies, Singapore) coupled with an Agilent 1260 Infinity Series High performance liquid chromatography (HPLC) system (Agilent, Waldbronn, Germany). The separation was performed with the Luna C18 column, size 4.6×150 mm, 5 µm (Phenomenex, USA) at a flow rate of 500 µL/min and the control temperature at 35 °C. The mobile phase A was water type I (Millipore, USA) and B was acetonitrile. Both phases contained 0.1 % (v/v) formic acid. The gradient elution mode started with 5 % solvent B to 95 % B linear gradient within 30 min and held on at this ratio for 10 min after that post-run 5 min for column equilibrium. The injection volume was 5 µL. The operating parameters for MS detection were as follows: drying gas (N_2) flow rate 10.0 L/min; temperature 350 °C; nebulizer pressure 30 psig; capillary 3500 V; skimmer 65 V; octapole RFV 750 V; and fragmentor voltage 250 V in negative mode and 100 V in positive mode. The mass range was set at m/z 100 - 1000 Da with a 250 ms/spectrum. The non-target MS/MS mode was set up at 3 collision energies of 10, 20, and 40 V using high purity nitrogen gas (99.999 %) as collision gas. All acquisition and analysis of the data were controlled by MassHunter Data Acquisition Software Version B.05.01 and MassHunter Qualitative Analysis Software B 06.0 respectively (Agilent Technologies, USA). Analysis of each sample was performed both in positive and negative ionization modes including non-targeted MS/MS mode to provide more information for structural identification. The m/z and fragmentation patterns of each compound were identified using metabolite databases such as the METLIN PCD/PCDL database (Agilent Technologies, USA), and public databases such as the Human Metabolome Database (http://www.hmdb.ca) and the Lipid maps Database (https://www.lipidmaps.org).

Data analysis and statistics

All experiments were carried out in triplicate except the LC-ESI-QTOF-MS/MS analysis. The data were presented as mean ± standard deviation.

Results and discussion

Total Phenolic Content and Antioxidant Activity

Folin-Ciocalteu method was used to determine the concentration of total polyphenol content. This method consists of determining the total polyphenol content through oxidation of phenolic compounds using a mixture of phosphotungstic and phosphomolybdic acids in base medium, producing blue acids of tungsten and molybdenum. This noni bio-fermented drink contained phenolic compound of 0.75±0.01 mg GAE/g. It means that there are less phenolic compounds in this drink. The scavenging activity of noni bio-fermented drink, as measured in the DPPH assay followed a linear positive dose-response. The scavenging

activity of noni bio-fermented drink displayed IC_{50} value at 14.94±0.76 µg/mL whereas Trolox at 8.47±0.16 µg/mL. The result present in **Figure 1** showed that the drink exhibited strong inhibition close to Trolox which was reflected in the low IC_{50}.

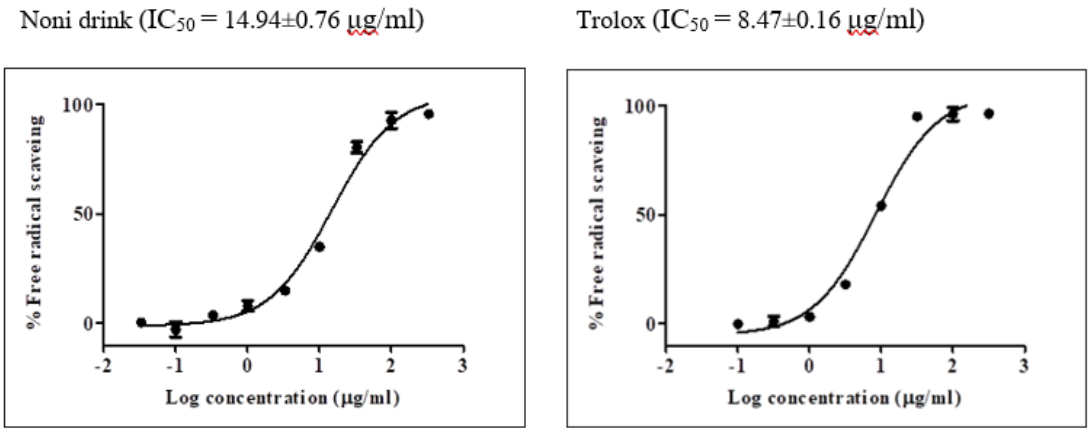


Figure 1 The inhibition curve of the noni bio-fermented drink and trolox at various concentrations against DHHP.

Phytochemical constituents in noni bio-fermented drink

Phytochemical screening for the presence of secondary metabolites in noni bio-fermented drink was characterized by LC-ESI-QTOF-MS/MS in both negative and positive ion modes. These compounds were identified on the basis of the accurate mass measurement (mass error), which was obtained by comparing the observed mass of their protonated $[M+H]^+$, deprotonated $[M-H]^-$, or other adduct ions to the theoretical exact mass in databases. Moreover, the fragment pattern (MS/MS) was used to confirm the structure of these compounds by their unique characteristics. The results demonstrated in **Figures 2 - 3** and **Table 1** revealed that 59 compounds were proposed but only 53 compounds were tentatively identified.

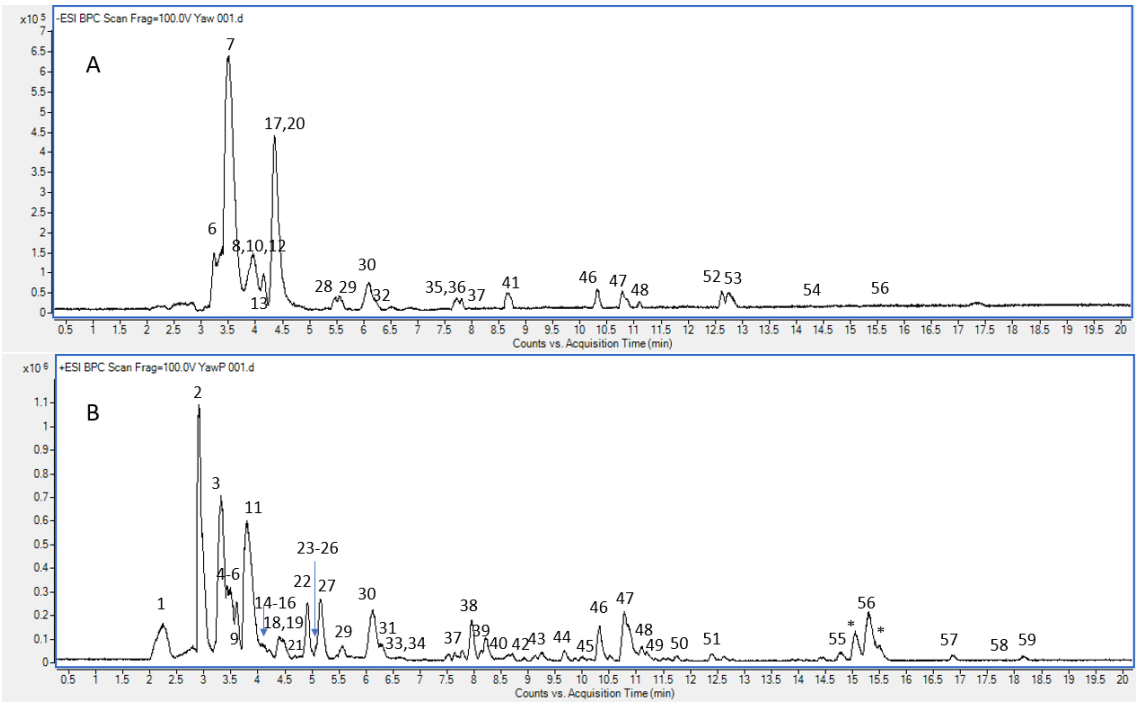


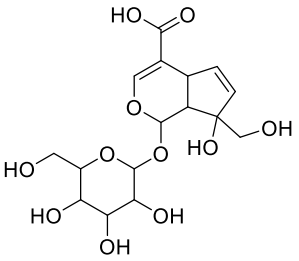
Figure 2 Base peak chromatogram (BPC) of noni bio-fermented drink output from A negative mode ESI(-), and B positive mode ESI(+). The number shown were the peak assignments as detailed in **Table 1**. * Peak contaminated from Nylon membrane.

Table 1 Tentatively identified compounds from *noni* bio-fermented drink.

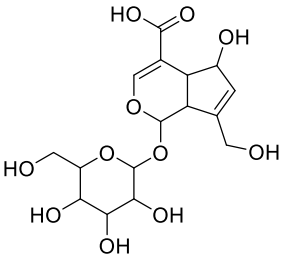
No.	RT (min)	m/z	Adduct	MS/MS	Tentative Identification	Formula	Error (ppm)
Amino acid and N-compound							
2	2.808	104.107	[M+H]+	60.0804,58.0647	2-Pentanol, 5-amino-, (R)-	C5H13NO	-0.09
5	3.435	160.0967	[M+H]+	88.0750,58.0647	Isovaleryglycine	C7H13NO3	0.75
9	3.605	118.0862	[M+H]+	72.0803	Norvaline	C5H11NO2	0.47
11	3.807	144.1015	[M+H]+	84.0801,58.0649	L-2-Amino-3-methylenehexanoic acid	C7H13NO2	2.81
14	3.967	280.1389	[M+H]+	262.1212,244.1106, 216.1169, 130.0825, 72.0785	N-(1-Deoxy-1-fructosyl)valine	C11H21NO7	0.64
15	4.121	247.1286	[M+H]+	86.0959,69.0686	Leucyl-Aspartate	C10H18N2O5	1
16	4.214	150.0583	[M+H]+	133.0305,104.0521, 61.0102,56.0489	L-Methionine	C5H11NO2S	0.17
18	4.403	203.1388	[M+H]+	86.0958,69.0690	Isoleucyl-Alanine	C9H18N2O3	1.08
19	4.469	158.1173	[M+H]+	98.0954,58.0646	3-(piperidin-3-yl) propanoic acid	C8H15NO2	1.61
22	4.916	132.1016	[M+H]+	86.0958	Leucine	C6H13NO2	2.31
23	4.938	189.1229	[M+H]+	132.1006,86.0959, 69.0684	Glycylleucine	C8H16N2O3	2.48
25	5.083	294.1543	[M+H]+	276.1420,230.1367, 144.1008,86.0960	N-(1-Deoxy-1-fructosyl)isoleucine	C12H23NO7	1.46
26	5.097	182.0809	[M+H]+	136.0748,91.0536	Tyrosine	C9H11NO3	1.48
27	5.161	132.1016	[M+H]+	86.0957	Isoleucine	C6H13NO2	2.31
29	5.558	128.0355	[M-H]-	82.0286,52.0197	Pyroglutamic acid	C5H7NO3	-1.43
	5.562	130.0498	[M+H]+	84.0440,56.0499	Pyroglutamic acid	C5H7NO3	0.53
38	7.957	166.0862	[M+H]+	120.0800,79.0536, 77.0382	Phenylalanine	C9H11NO2	0.33
39	8.135	231.1703	[M+H]+	86.0948	Isoleucyl-Valine	C11H22N2O3	0.08
42	8.71	231.1703	[M+H]+	86.0960,72.0805	Valylleucine	C11H22N2O3	0.08
43	9.13	265.1548	[M+H]+	120.0798	Phenylalanylvaline	C14H20N2O3	-0.49
50	11.749	231.1121	[M+H]+	214.0817,188.0674, 158.0939,130.0631, 74.0223	1-Methyl-1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid	C13H14N2O2	3.05
Organic acid							
4	3.375	180.0865	[M+NH4]+	127.0380,85.0277	2-Hydroxy-2-ethylsuccinic acid	C6H10O5	0.83
7	3.509	195.0508	[M-H]-	129.0184,75.0085	Gluconic acid	C6H12O7	1.16
8	3.555	193.0351	[M-H]-	129.0183,75.0087, 59.0139	Pectic acid	C6H10O7	1.43
10	3.77	191.0564	[M-H]-		Quinic acid	C7H12O6	-1.51
13	3.944	193.0361	[M+HCOO]-	103.0030,59.0137	Citramalic acid	C5H8O5	-3.75
17	4.363	133.0143	[M-H]-	115.0029,71.0138	Malic acid	C4H6O5	-0.4
20	4.5	191.0201	[M-H]-	111.0080,73.0292	Citric acid	C6H8O7	-1.96
28	5.473	191.0198	[M-H]-	111.0076,87.0081, 57.0345	Isocitric acid	C6H8O7	-0.39
32	6.19	117.0195	[M-H]-	73.0294	Succinic acid	C4H6O4	-1.43
33	6.276	197.08	[M+H]+	179.0693,107.0486, 79.0536	3-(3-hydroxy-4-methoxyphenyl)propanoic acid	C10H12O4	4.24
35	7.723	173.0093	[M-H]-	85.0292	Cis-Aconitic acid	C6H6O6	-0.8
36	7.723	129.0196	[M-H]-	85.0295	Mesaconic acid	C5H6O4	-2.08
41	8.674	173.0094	[M-H]-	85.0292	trans-Aconitic acid	C6H6O6	-0.8

No.	RT (min)	m/z	Adduct	MS/MS	Tentative Identification	Formula	Error (ppm)
Iridoid glycoside							
30	6.091	389.1092	[M-H]-	209.0421,165.0540 ,89.0239	Monotropein	C16H22O11	-0.68
	6.124	408.1492	[M+NH4]+	211.0587,193.0483, 175.0378,147.0430, 119.0482,85.0271	Monotropein	C16H22O11	2.05
37	7.808	413.105	[M+Na]+		Deacetylasperulosidic acid	C16H22O11	1.05
	7.812	389.1086	[M-H]-	209.0430,183.0639, 165.0539,89.0235	Deacetylasperulosidic acid	C16H22O11	-0.68
46	10.319	455.115	[M+Na]+	411.1153,275.0505	Asperulosidic acid	C18H24O12	2.19
	10.326	431.1187	[M-H]-	251.0531,165.0542, 119.0346,59.0139	Asperulosidic acid	C18H24O12	1.86
Coumarin							
53	12.767	177.0195	[M-H]-	133.028	Aesculetin	C9H6O4	-0.95
56	15.312	193.0489	[M+H]+	133.026	Scopoletin	C10H8O4	3.29
	15.316	191.0349	[M-H]-	176.0097,148.0146, 104.0254	Scopoletin	C10H8O4	0.43
Flavonoids							
24	4.999	226.1281	M+NH4	145.0481,85.0276	Ethyl beta-D-glucopyranoside	C8H16O6	1.83
31	6.125	803.2189	[M+H]+		Spinacetin 3-(2"-apiosylgentiobioside)	C34H42O22	6.41
47	10.811	403.157	[M+Na]+	271.1127	Prenyl arabinosyl-(1->6)-glucoside	C16H28O10	1.16
	10.803	425.1657	[M+HCOO]-	379.1560,149.0441, 89.0239	Prenyl arabinosyl-(1->6)-glucoside	C16H28O10	1.76
48	11.103	271.1146	[M+Na]+	98.9729	Prenyl glucoside	C11H20O6	2.25
	11.103	293.1239	[M+HCOO]-	112.9834,68.9948	Prenyl glucoside	C11H20O6	0.99
51	12.405	469.1305	[M+H]+	413.2575,317.0797, 183.0621,175.0328, 123.0423	2-(3,4-dihydroxyphenyl)-8-[3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]-3,4-dihydro-2h-1-benzopyran-3,5,6,7-tetrol	C21H24O12	7.57
54	14.443	375.108	[M+HCOO]-	329.0983,137.0232	7-O-Methyl-3,9-dihydropunctatin	C18H18O6	1.44
Sulfur compound							
1	2.224	185.0662	[M+H]+	127.0375,83.0335, 68.9828	2-Sulfanylpropan-1-ol;3-sulfanylpropan-1-ol	C6H16O2S2	1.35
21	4.694	123.0472	[M+H]+	105.0360,77.0045	Thiodiglycol	C4H10O2S	1.84
Miscellaneous							
3	3.32	198.0971	[M+NH4]+	145.0485,85.0278	Hexose	C6H12O6	0.57
6	3.466	360.1498	[M+NH4]+	145.0486,127.0381, 85.0278	Sucrose	C12H22O11	0.66
	3.364	341.1061	[M-H]-	179.0541,89.0237	Sucrose	C12H22O11	8.31
12	3.849	177.0408	[M-H]-	99.0077,59.0135	L-Gulonolactone	C6H10O6	-1.91
44	9.253	127.0408	[M+H]+	109.0278,81.0325, 53.0381	benzene-1,2,4-triol	C6H6O3	-14.4
52	12.629	441.0115	[M+HCOO]-	396.9990,395.0007	Sucralose	C12H19Cl3O8	2.84
55	14.789	116.1067	[M+NH4]+	59.048	4-Methyl-4-penten-2-one	C6H10O	2.5
58	17.748	159.1027	[M-H]-	59.0135	8-Hydroxyoctanoate	C8H16O3	-0.2
34	6.28	451.1563	[M+H]+	237.0718,163.0727	Unidentified		
40	8.223	421.1676	[M+H]+	289.1245	Unidentified		
45	9.675	223.181	[M+H]+	129.1376,95.0484, 67.0538	Unidentified		

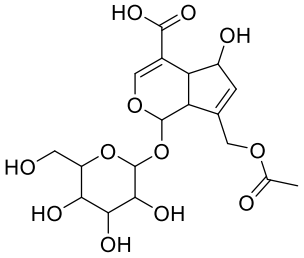
No.	RT (min)	m/z	Adduct	MS/MS	Tentative Identification	Formula	Error (ppm)
49	11.203	419.1509	[M+H]+	317.0811,229.0661, 185.0378,73.0268	Unidentified		
57	16.901	383.398	[M+H]+	325.2068,203.0493	Unidentified		
59	18.173	329.1518	[M+H]+	214.9136,140.9145, 84.9578	Unidentified		



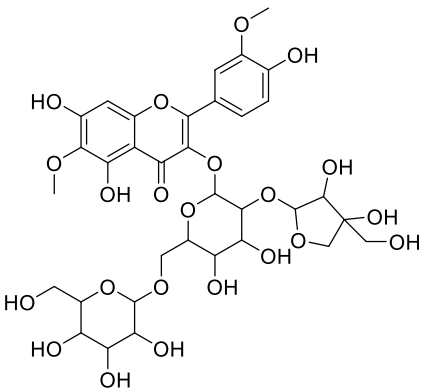
30.Monotropein
Chemical Formula: $C_{16}H_{22}O_{11}$
Exact Mass: 390.12
Molecular Weight: 390.34



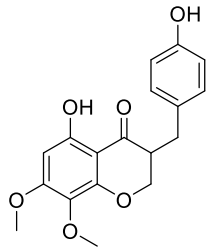
37. Deacetylasperulosidic acid
Chemical Formula: $C_{16}H_{22}O_{11}$
Exact Mass: 390.12
Molecular Weight: 390.34



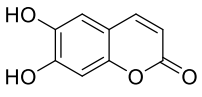
46. Asperulosidic acid
Chemical Formula: $C_{18}H_{24}O_{12}$
Exact Mass: 432.13
Molecular Weight: 432.38



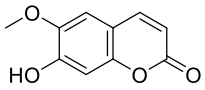
31.Spinacetin 3-(2''-apiosylgentiobioside)
Chemical Formula: $C_{34}H_{42}O_{22}$
Exact Mass: 802.22
Molecular Weight: 802.69



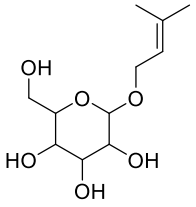
54.7-O-Methyl-3,9-dihydropunctatin
Chemical Formula: $C_{18}H_{18}O_6$
Exact Mass: 330.11
Molecular Weight: 330.34



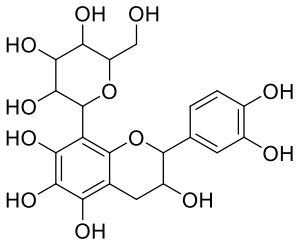
53.Aesculetin
Chemical Formula: $C_9H_6O_4$
Exact Mass: 178.03
Molecular Weight: 178.14



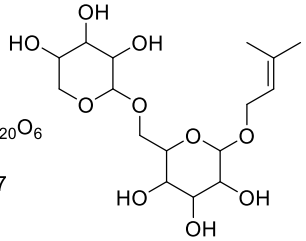
56.Scopoletin
Chemical Formula: $C_{10}H_8O_4$
Exact Mass: 192.04
Molecular Weight: 192.17



48.Prenyl glucoside
Chemical Formula: $C_{11}H_{20}O_6$
Exact Mass: 248.13
Molecular Weight: 248.27



51.2-(3,4-dihydroxyphenyl)-8-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-3,4-dihydro-2h-1-benzopyran-3,5,6,7-tetrol
Chemical Formula: $C_{21}H_{24}O_{12}$
Exact Mass: 468.13
Molecular Weight: 468.41



47.Prenyl arabinosyl-(1->6)-glucoside
Chemical Formula: $C_{16}H_{28}O_{10}$
Exact Mass: 380.17
Molecular Weight: 380.39

Figure 3 Some chemical constituents found in noni bio-fermented drink.

The noni bio-fermented drink contained numerous amino acids and dipeptides (**5**, **9**, **15**, **16**, **18**, **22**, **23**, **26**, **27**, **29**, **38**, **39**, **42** and **43**) and some conjugated to sugar atoms (**14** and **25**). Fructosamine (compounds **14** and **25**) were intermediate of the Maillard reaction, which responsible for specific aroma, taste, and color formation in thermally processed or dehydrated foods [13]. Compounds **2**, **11**, **19** and **50** contained nitrogen atoms in their molecules and categories as N-compound. Amino acids were used in the synthesis of protein in the body such as muscle and skin. The variety of small organic acids were found (**4**, **7**, **8**, **10**, **13**, **17**, **20**, **28**, **32**, **33**, **35**, **36** and **41**) because of the fermented process. These compounds could be responsible for the characteristic taste of this drink. The iridoid glycoside; monotropein (**30**), deacetylasperulosidic acid (**37**), and asperulosidic acid (**46**) were proposed. Deacetylasperulosidic acid was reported as antioxidant activity in vivo and in human trials [14]. The coumarin aesculetin (**53**) and scopoletin (**56**) have been identified. Aesculatin or esculetin is a bifunctional antioxidant. It prevents and counteracts the oxidative stress and neuronal death induced by Amyloid Protein in SH-SY5Y Cells [15].

Furthermore, flavonoids glycoside Spinacetin 3-(2"-apiosylgentiobioside) (**31**), 2-(3,4-dihydroxyphenyl)-8-[3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]-3,4-dihydro-2h-1-benzopyran-3,5,6, 7-tetrol (**51**) were assigned. 7-O-Methyl-3,9-dihydropunctatin (**54**) was a homoisoflavonoids, which one additional carbon atom (C9) in their skeleton that differed from flavonoid and isoflavonoids. This compound was inactive to cytotoxic activities using the MDA-MB-435 (melanoma) and HT-29 (colon) cancer cell lines and antimicrobial activity using a collection of bacteria and fungi [16]. 2-Sulfanylpropan-1-ol;3-sulfanylpropan-1-ol (**1**), L-Methionine (**16**) and Thioglycol (**21**) contained sulfur atom in their molecule these compounds might be the cause of unpleasant smell of this drink. Benzene-1,2,4-triol (**44**), this compound when exposed to air it produced a black insoluble solid [17]. Sucralose (**52**) is an artificial sweetener used as sugar substitute during the final adjusted to taste stage.

The dry slice noni fruit decoction exhibited compound differ from bio-fermented drink. Mostly, they contained a lot of flavonoids glycoside and phenolic compounds. Iridoid glycosides. Sanshiside D was reported in the decoction sample but did not find in this drink [18]. Both of samples originated in Thailand. These data might be reflected the processing and formulation on the phytochemical constituents.

Conclusions

The phytochemical profiling established in our study serves as a useful method for identifying the biological compounds and phytonutrient regarding efficacy and safety. As a result, noni bio-fermented drink contained a certain number of flavonoids and Iridoid glycoside which suggest antioxidant potential. Furthermore, the active compounds can be selected as a marker for quality control. The noni bio-fermented drink from Tub Yai Chiang sub district has its own characteristic with diversity of chemical constituents that may not be applicable to any other samples. The chemistry behind these compounds in noni bio-fermented drinks is remarkable as a potential new source of health drink.

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