Antioxidant activity of three different preparations of noni fruit juice (ရဲယို)

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Introduction

- ***Morinda citrifolia*** **is the scientific name of the noni plant.**
- **Noni fruit (Ye Yo*)* grows native in Panama.**
- ***Morinda citrifolia*** **is a plant or tree that grows in hot, tropical environments (1).**
- **Noni is among the traditional medicinal plants used as remedies in Myanmar.**

- **Nowadays, noni manufacturers produce various form of noni juice from ripe noni fruits as dietary supplements and herbal drug in market used to treat diseases in many countries and region (2, 3).**

- **fruits are used as emmenagogues, blood purifiers, antiemetic agents and tonic supplements and for digestive disorders, tuberculosis, urinary-tract ailments, stimulation of appetite and central nervous system, hypertension, diabetes and depression**

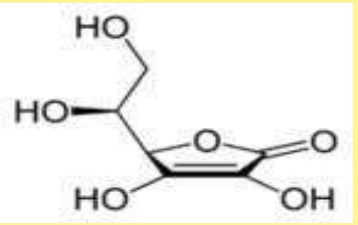
- **helps the immunological system work better (3).**

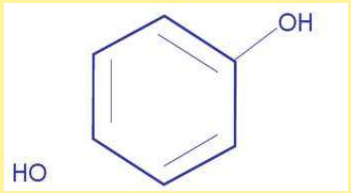
- **It has been used by people with immuno-compromised diseases like chronic fatigue syndrome to boost the immune system function.**

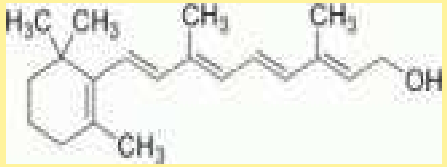
- **In smokers, the results showed that total cholesterol and triglycerides improved after one month of drinking noni juice (4).**

Noni fruit is an excellent source of nutrients including

- **vitamin C**
- **vitamin A**
- **niacin**
- **potassium**
- **calcium**
- **sodium.**



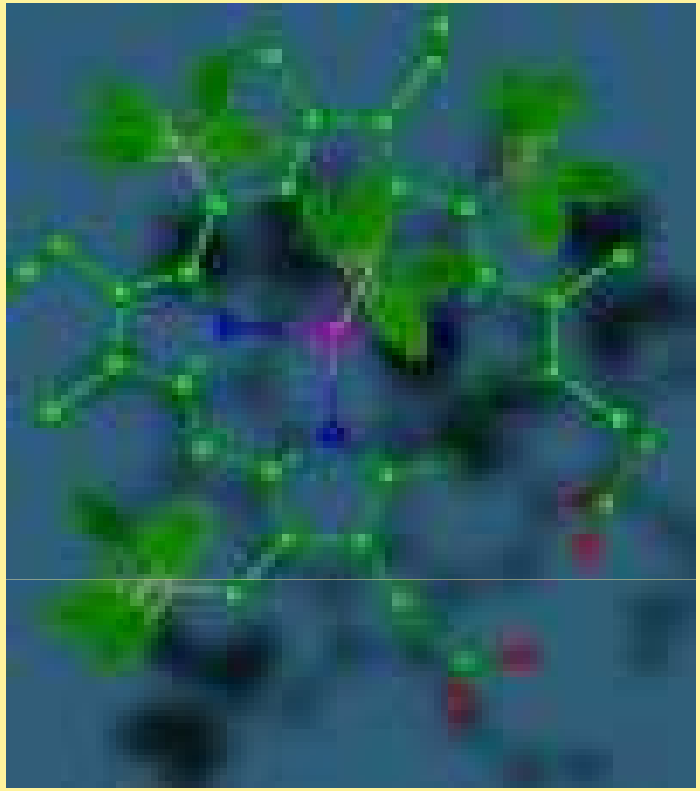




It has identified the presence of

- **10 essential vitamin**
- **7 dietary minerals**
- **18 amino acid**
- **mixture of anthraquinones**
- **organic acids**
- **xeronine (5).**

- **Dietary foods contain a wide variety of free radical-scavenging antioxidants; for example, flavonoids and antioxidative vitamins such as ascorbic acid and α-tocopherol.**



- **These compounds are particularly rich in vegetables, fruits, tea and wine.**

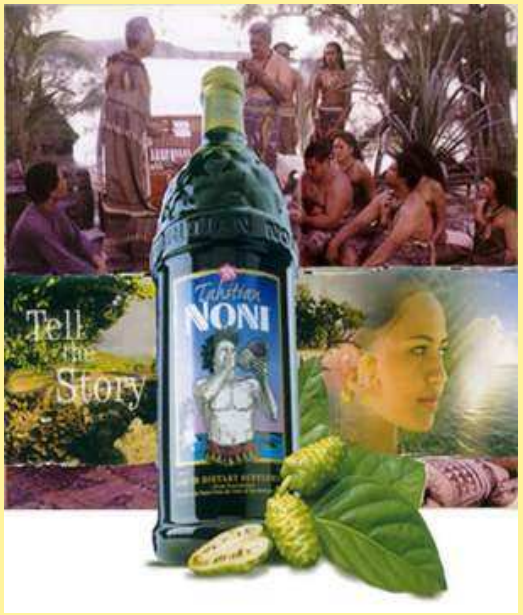
- **On the other hand, colorimetry with DPPH, a stable free radical, has been reported as a simple method for evaluation of the free radical- scavenging activity (6).**
- **In this study, the free radical-scavenging activity in three different preparations of noni fruit juice was determined by using *in vitro* DPPH method which is simple and rapid**
- **The antioxidant activity of noni fruit was found to be quite all dependent on polyphenols: including vitamin C, vitamin A, flavonoid and polyphenol.**

- **In fermentation method, noni fruit was fermented under sunlight for 6 weeks.**
- **Expressed juice and boiling juice are commonly used for traditional medicine.**

- **High temperature used in boiling may lead to phenolic depletion, while polyphenol are mainly degraded by exposure to large amounts of oxygen (7).**
- **Therefore, this study is to evaluate the antioxidant activity of three types of noni fruit juice for antioxidant capacity.**

General objective

- To investigate the antioxidant activity of three different preparations of noni fruit juice



Specific objectives

- To determine antioxidant properties, total phenolic content and ascorbic acid content of three different preparations of noni fruit juice
- To determine acute toxicity study of three different preparations of noni fruit juice on mice

Sample size

Five serial concentrations of standard and test samples from three types of noni fruit juice were determined three times.

Study period

November 2012 to May 2013

Study Design

Descriptive study for chemical test
Control parallel design study for acute toxicity test.

Study site

Pharmacology Research Division
Laboratory Animal Service Division, DMR (LM)

Materials and Methods

Materials

- Ripe noni fruits
- 97% Ethanol (Local)
- Micropipette (20-200 ul)
- 1, 1-diphenyl -2-picrylhydrazyl (DPPH)
- Sodium carbonate (AnalaR, BDH)
- 2,6 dichlorophenolindophenol sodium salt (AnalaR, BDH)
- Oxalic acid
- Folin - Ciocalteu reagent
- Standard ascorbic acid
- Quercetin
- Mice (DDY strain)
- UV Spectrophotometer (UV-1601, SHIMADZU)

Methods

Sample collection

In this study, mature white hard noni fruit were collected from Yangon area during September 2012 to May 2013. When ripen soft of collected noni fruit were tested.

(1) Preparation of three types of noni fruit juice (8,9)

Noni fruit juice was prepared in three ways.

(i) Fermented juice: (အချဉ်ဖေါက် ဖျော်ရည်)

- **Firstly, wash ripened noni fruits with water, then put all the fruits inside a sterile airtight glass container. Put the container outside in sunshine for 6 weeks. During this time the fruit was ferment and separate on the bottom of the container.**

(ii) Expressed juice: (သတ္တု ရည်)

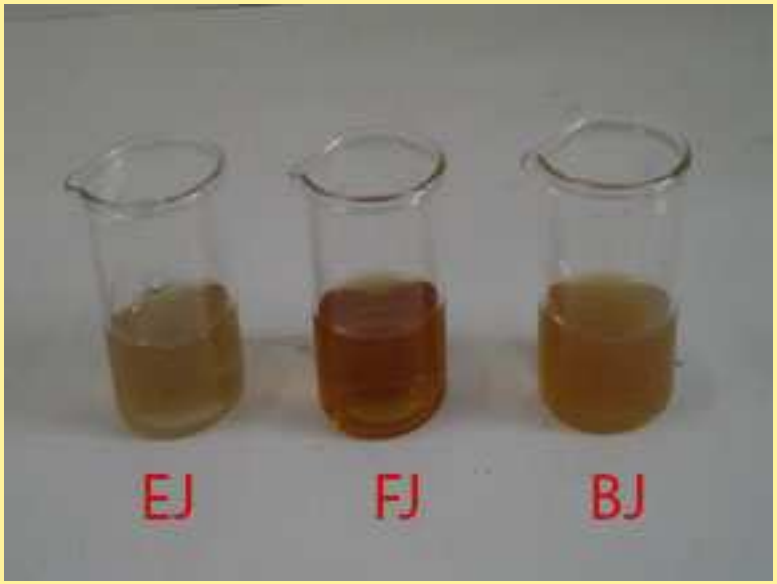
- **Expressed juice is the liquid product obtained by crushing and blending fresh noni fruits. These juices were squeezed, filtered and then centrifuged at 3000 rpm for 30 minutes. Expressed juice is the freshly squeezed unfermented sweeter version.**

(iii) Boiling juice: (ပြုတ် ရည်)

❖ **Freshly ripened noni fruits with distilled water 1:2 (w/v) were boiled using high temperature for 30 minutes to evaporate all the water. Boiled fruits was squeezed, filtered and then centrifuged at 3000 rpm for 30 minutes. The juice was collected in the sterilized container to be tested.**

(2) Determination of physico-chemical characterization

Determination of pH value in three different preparations of noni fruit juice were done by pH meter (pH-700, Eutach) and sugar content was tested by refractometer (Pal-1, Atago).



(3) Qualitative identification by thin layer chromatography (10)

- Ascorbic acid and polyphenol compounds were identified in three different preparations of noni fruit juice by thin layer chromatography.
- Solvent system was using n-butanol : acetic acid : water (4:1:5) on silica gel GF_{254} precoated TLC plate.
- Visualizing reagent was used 10% ferric chloride spray for polyphenol compounds and ascorbic acid was seen under short wave ultraviolet light.

(4) Quantitative determination of total phenol content (11, 12)

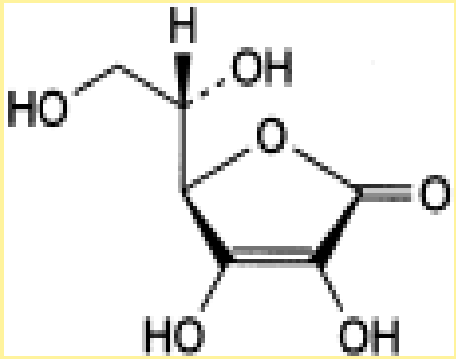
- The total phenolic contents of three types of noni fruit juice were measured with the Folin-Ciocalteu regent.
- Firstly, 1.6 ml of diluted noni fruit juices and 100 µl of Folin-Ciocalteu regent were mixed, then 300 µl of saturated Na_2CO_3 (20%) were added.
- After the solution was incubated 40°C for 30 min, the absorbance of the solution was measured at 765 nm with the UV Spectrophotometer.
- Total phenolic content of noni fruit juices were calculated from quercetin standard curve.

(5) Quantitative determination of ascorbic acid content

(10, 13)

Ascorbic acid content of three different preparations of noni fruit juices were determined by Unani method with UV spectrophotometer. The diluted fruit juice were mixed with 1: 9 of 2,6 dichlorophenolindophenol solution. Exactly 15 second after adding the dye, the solution was measured at 520 nm by UV Spectrophotometer. The ascorbic acid content of noni fruit juices were calculated based on the ascorbic acid standard curve.

Vitamin C



(6) Determination of antioxidant activity (12,14,15)

Serial dilutions of three different preparations of noni fruit juice were diluted with 50% ethanolic solution. Test samples and 60 µM DPPH solution were mixed (1:1) vigorously by a vortex mixer. All solutions were allowed to stand at room temperature in dark place for 30 minutes, after which measurement of absorbance were done at 517 nm using UV Spectrophotometer (UV-1601, Shimadzu). Absorbance measurements were calculated in formula, results were shown in percent inhibition.

(7) Acute toxicity study on animal model (16)

Acute toxicity study of three different preparations of noni fruit juice were done according to the method of Litchfield & Wilcoxon (1949). Both sex of hundred albino mice in 10 groups (10 mice/group) were given orally three preparations of noni fruit juice at the dose of 17.0, 33.5 and 67.0 ml/kg body weight per day. The control group received 10ml/kg body weight of distilled water. All the animals were kept under observation for toxic effect for two weeks.

Results and Discussion

Physico-chemical characterization of three different preparations of noni fruit juice were shown in Table (1).

Table (1) Physico-chemical characterization of noni fruit juices

Sample	Yield percent (%)	Soluble matter (%)	pH value	Sugar content (%)
Expressed juice	40.0	9.6	3.8	9.5
Fermented juice	48.0	7.3	3.6	8.9
Boiling juice	43.0	9.7	3.9	9.1

Ascorbic acid was identified in EJ, FJ and BJ on thin layer chromatogram observed R_f value (0.67), dark color under short wave ultraviolet light compare with standard compound.

Thin layer chromatogram of **phenolic compounds** in EJ, FJ and BJ were showed bluish black color after spray with 10% Ferric chloride reagent. These results were shown in Figure (1).

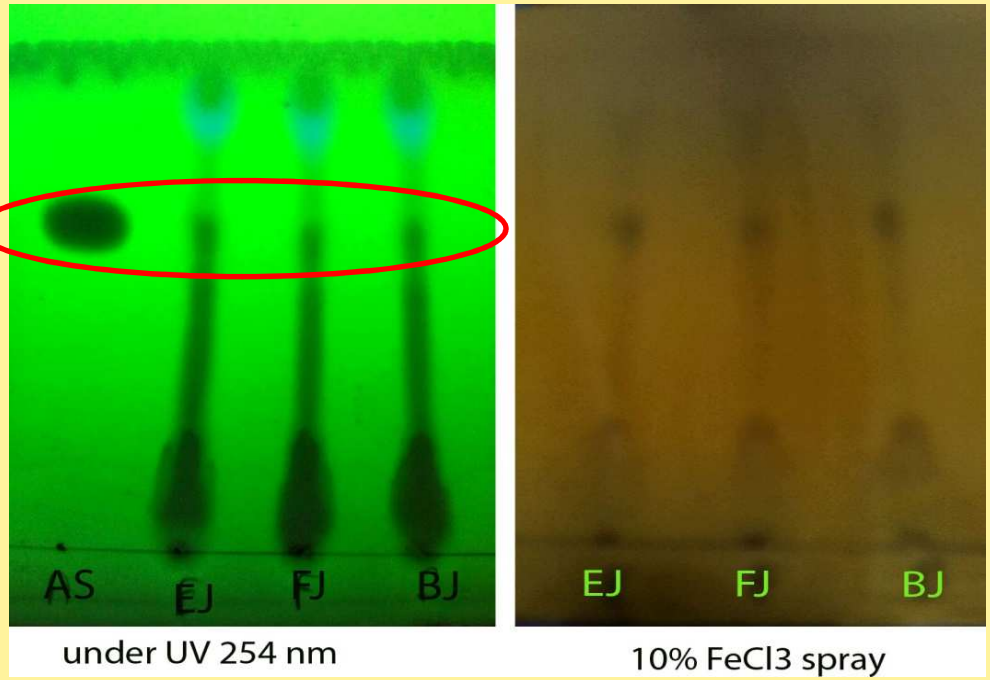


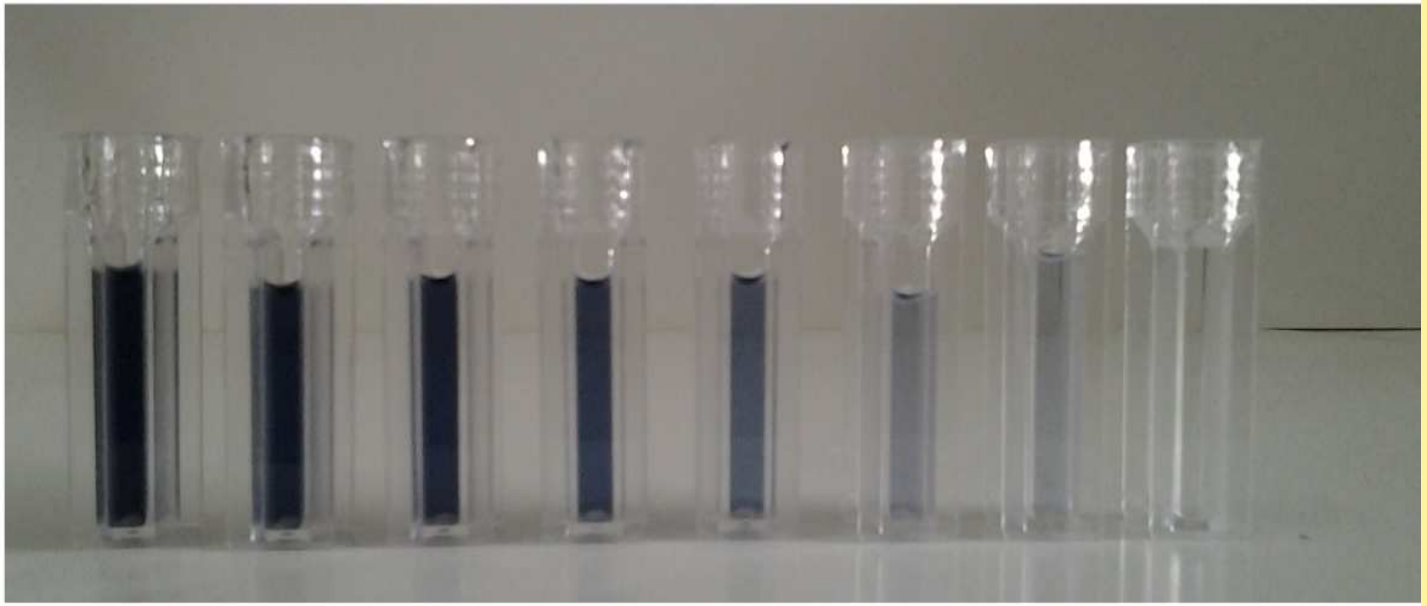
Figure (1) Thin layer chromatogram of ascorbic acid and phenolic compounds

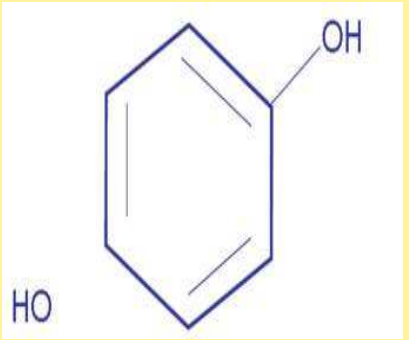
The total phenols content and ascorbic acid content in EJ, FJ and BJ of noni fruit were shown in Table (2).

Table (2). Total phenol content and ascorbic acid content of noni fruit juices

Sample	Total phenol (mg/ml)	Ascorbic acid (mg/ml)
Expressed juice	4.1 ± 0.1	1.12 ± 0.1
Fermented juice	2.77 ± 0.7	0.77 ± 0.1
Boiling juice	2.23 ± 0.2	0.48 ± 0.1

- **Expressed juice of noni fruit exhibited total phenols content and ascorbic acid content significantly higher than those of fermented juice and boiling juice.**





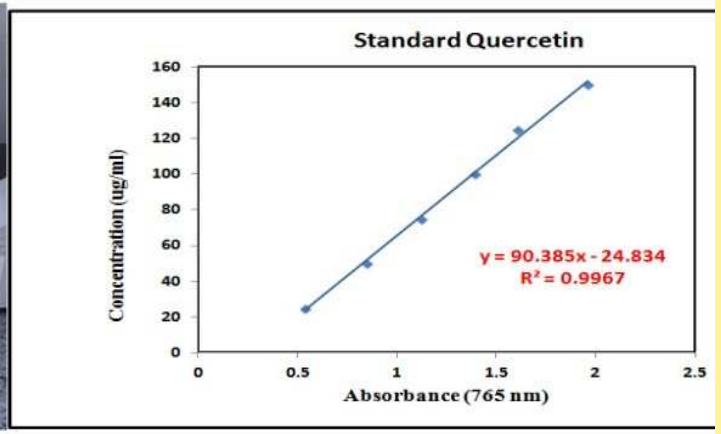
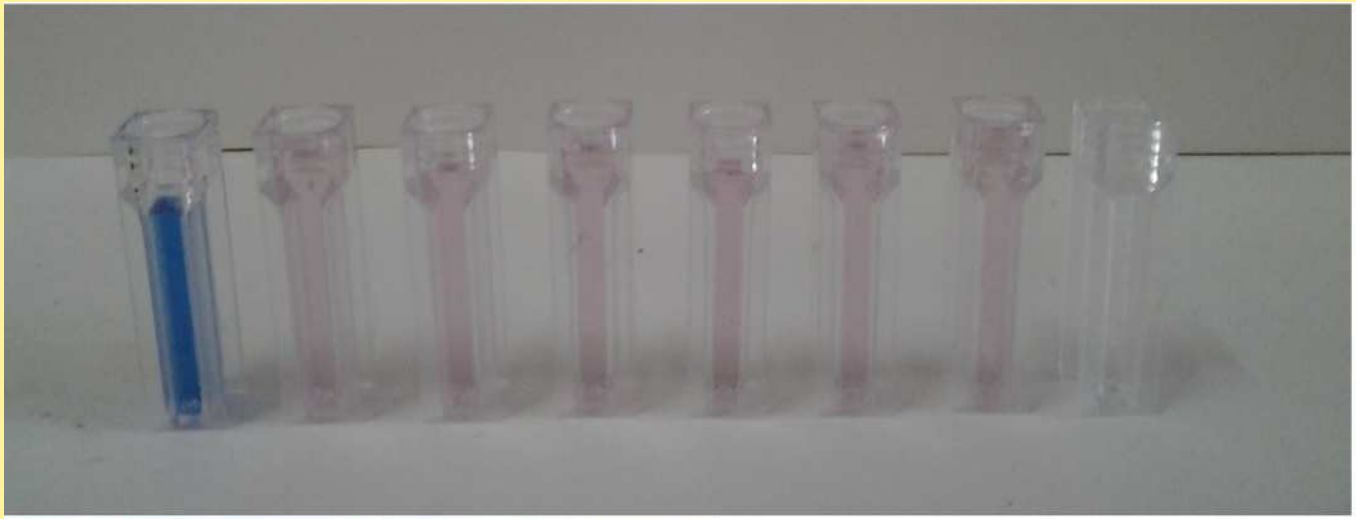
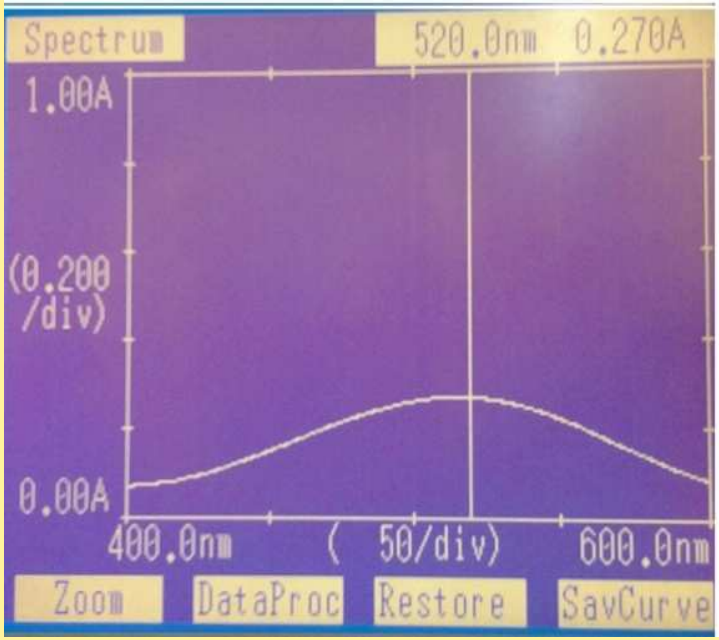
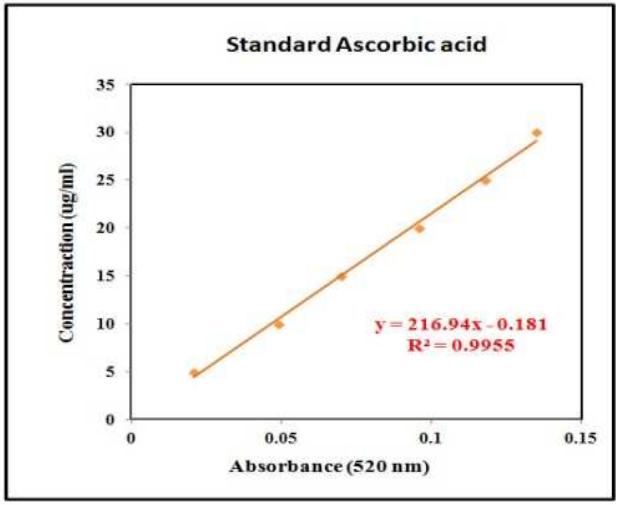


Figure (2) Figure of quantitative determination of total phenol content by UV Spectrophotometer







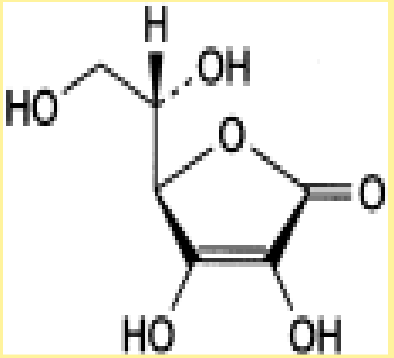


Figure (3) Figure of quantitative determination of ascorbic acid content by UV spectrophotometer

- The loss of total phenol contents and ascorbic acid content in fermented juice by fermentation process were 32.5% and 31.3% when fermented under sunlight for 6 weeks.
- In fermentation process, fermented juice was subjected to strong UVA and UVB light and temperature range from 28ºC to 32ºC, which is a typical tropical environment.
- In boiling juice, the loss of total phenol concentration and ascorbic acid concentration were 45.6% and 57.1% when boil at high temperature for 30 min.

- **Three different preparations of noni fruit juices (EJ, FJ, BJ) 4 ug/ml were possessed antioxidant activity and results of percent inhibition were showed in Table (3) and Figure (4).**
- **In this study, free radical scavenging activity of fermented juice and boiling juice were 13.7% and 21.3% lower than expressed juice.**

Table (3) Percent inhibition of three different preparations of noni fruit juice

No.	Noni fruit juice Conc: (µg/ml)	% inhibition 0.25µg/ml	0.5 µg/ml	1.0 µg/ml	2.0 µg/ml	4.0 µg/ml
1	Expressed juice	59.2	61.4	65.1	76.3	83.8
2	Fermented juice	56.4	62.9	67.6	69.8	72.3
3	Boiling juice	42.7	46.1	54.5	63.2	66.0
4	Ascorbic acid	51.7	66.98	75.4	91.3	97.2

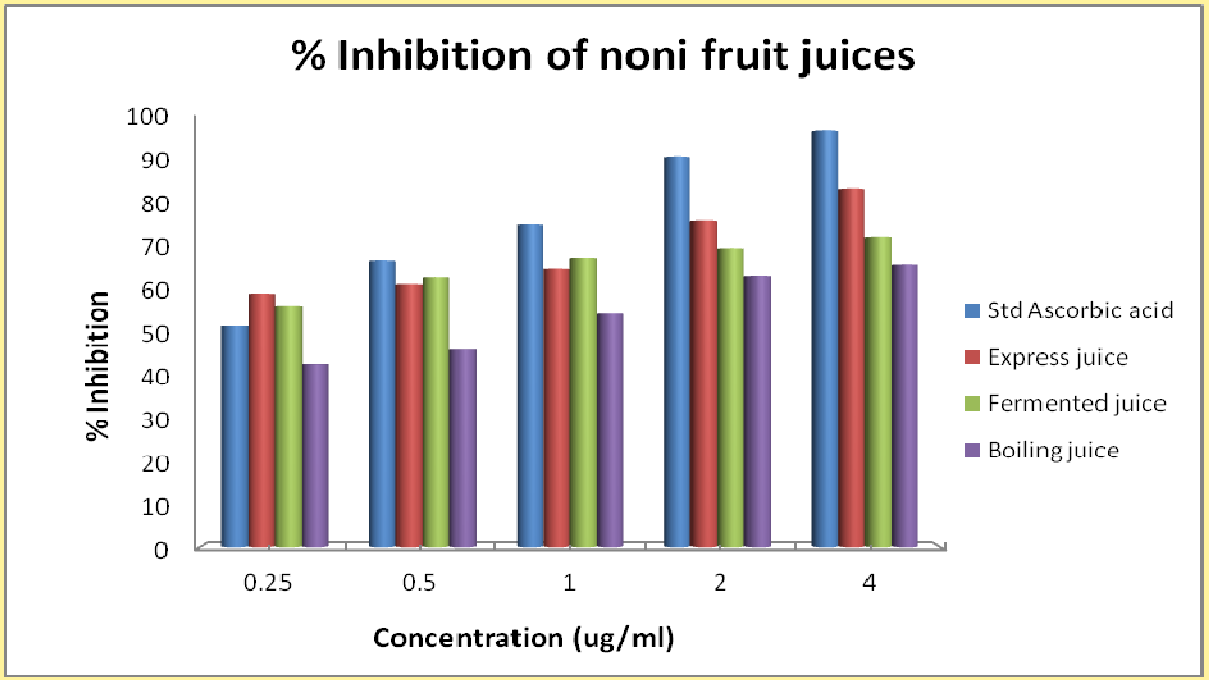


Figure (4) Figure of antioxidant activity of three types of noni fruit juice

Table (4) Acute toxicity study of three different preparations of noni fruit juice

Group	Number of mice/group	Diet	Dosage of juice (ml/kg)	Observed period	Ratio of dead and tested	Percentage of death
control	10	Stock diet and distilled water	Distilled	Two weeks	0/10	0
EJ/ FJ/ BJ	10	ditto	17	Two weeks	0/10	0
	10	ditto	33.5	Two weeks	0/10	0
	10	ditto	67	Two weeks	0/10	0

- **In acute toxicity test, toxic effect was not found in all three different preparations of noni juice in maximal permissible dose.**
- **Therefore, the median lethal dose (LD_{50}) was more than 67 ml/kg body weight that is equal to 42 ml/50kg body weight human dose.**

- **From the results, antioxidant activities of different preparations of noni fruit juice depend on total phenol content and ascorbic acid content.**

- **Expressed juice has the highest antioxidant activity, ascorbic acid content and total phenol content.**

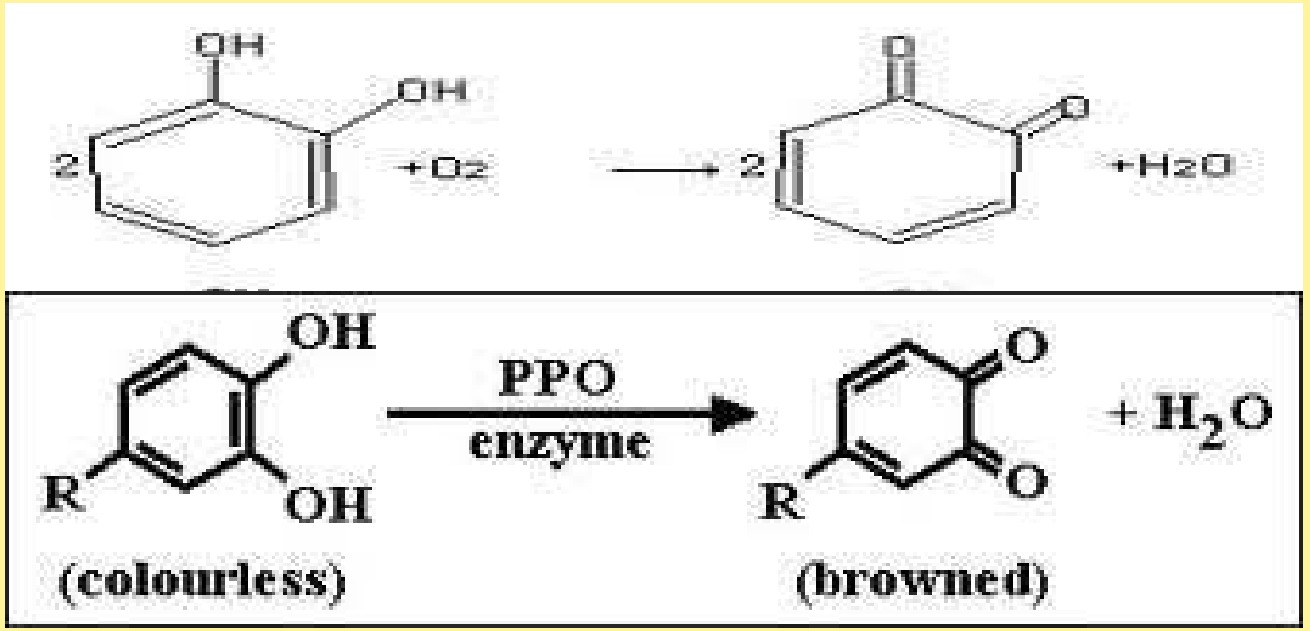
- **The boiling juice of noni fruit showed lowest antioxidant activity due to high temperature of the juice processing method.**

- **Fresh noni fruit is a good source of antioxidants and phenolic. But traditional fermentation practice and storage at room temperature dramatically decreased its free radical scavenging activity. Noni fruit juice lost a significant percentage of radical scavenging activity within 1 week when stored at 24ºC but not within 1 week at 4ºC or within 2 weeks at -18ºC (17).**

- **Ascorbic acid is unstable, especially in alkali solution, readily undergoing oxidation even by atmospheric oxygen, the change being accelerated by light and heat (18).**
- **The loss of vitamin C of *Phyllanthus emblica* L. fruit by different drying processes were 86.58 % when dried in shade for four days, 80.90% when dried in oven at 75°C for four hours, 71.33% when directly in sunshine for four hours, 66.2% dried in oven at 50°C for four and half hours, and 47.3% drying in microwave oven for six minutes respectively (10).**

- **In this study, the highest ascorbic acid content was 1.12 mg/ml in fresh expressed juice of noni fruit. No high temperature was used during the fruit juice process.**
- **During fermentation process of fermented juice there was 31.3% decrease in ascorbic acid content due to low temperature for 6 weeks.**
- **In boiling juice, there was 57.1% decrease ascorbic acid concentration due to high temperature for 30 minutes.**
- **This finding indicates that the loss of ascorbic acid content in fruit juice also depend on temperature.**

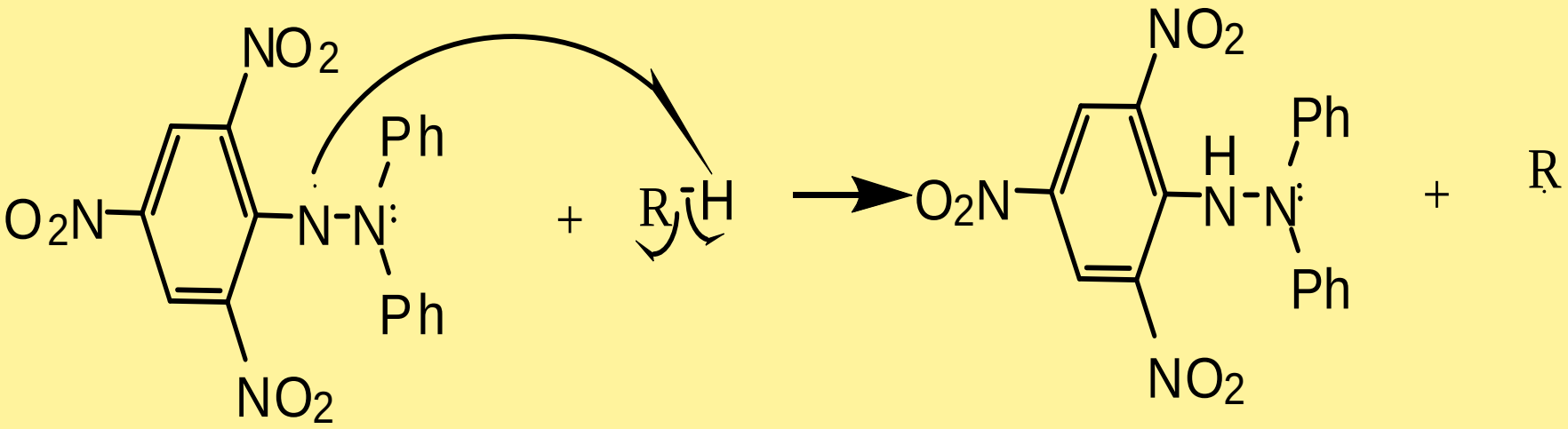
- **Kejitan Trost., *et.al*. (2008) reported that during 9 months of blueberry nectar storage, there was 3 – 9.9% decrease in polyphenol concentrations and antioxidant activity decreased by 20.9 – 24.2% (19).**
- **Phenolic compounds have an antioxidant activity, which is correlated to decreases in many diseases (19).**
- **The higher antioxidant activity was observed in the expressed juice of noni fruit than fermented juice and boiling juice.**
- **This study showed that changes in total phenol concentration and ascorbic acid concentration caused change in antioxidant activity .**



(Degradation of polyphenol)

- **These equations show the degradation of polyphenol compound due to oxidation from environment effect (20).**

- **The principle of the DPPH method is that in the presence of a stable free radical, (DPPH), an antioxidant donates a hydrogen atom to quench the stable free radical. This method can be applied either when the antioxidant in its pure form or in a mixture.**



1, 1-diphenyl -2-picrylhydrazyl (DPPH)
[Purple color]

[Pale yellow color]

- **The DPPH radical scavenging assay determined free antioxidants in noni juice, whereas the assay of total phenols with Folin - Ciocalteu reagents determined both free phenolic and bound phenolic in noni juice (21).**
- **Non phenolic antioxidants such as ascorbic acid exhibit rapid reactions to the DPPH radical (22).**

- **in the present study indicated that the polyphenols present in the fruit juice are destroyed (oxidized) to some extent during the processing method in sunlight and/or heating.**

- **As polyphenolic antioxidants are known to positively influence human health, stability determination is crucial.**

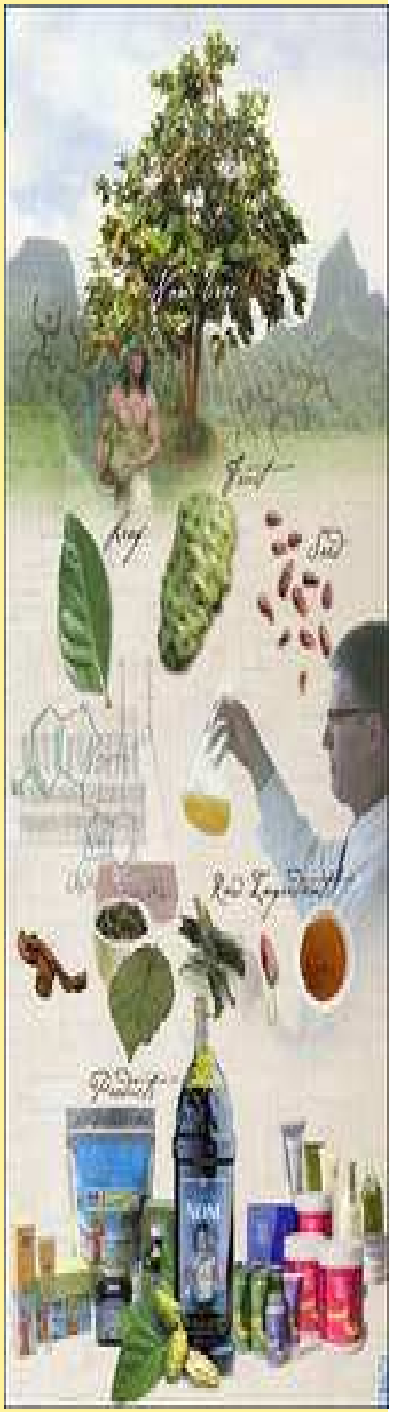
- **Increase of temperature, degradation of polyphenol was higher.**

- **The antioxidant activities of fermented juice and boiling juice are affected by the temperature during the noni juice preparation method.**

- **This study showed that antioxidant activity depends on ascorbic acid content and total phenol content of three different preparations of noni fruit juice.**

Conclusion

- It can be concluded that freshly expressed juice contain high amount of ascorbic acid content and total phenol content than other fermented juice and boiling juice.
- These results suggested that expressed juice of noni fruit provided antioxidant properties and offered effective protection from free radicals .
- It also supported that noni fruit is a promising source of natural antioxidants.
- Therefore, prevention of the thermal degradation of polyphenol compound in noni fruit juice is needed for antioxidant activity.
- Fresh expressed juice rather than fermented juice and boiling juice is recommended for its substantial antioxidant properties.



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Thank You