Comparative Evaluation on Rutin Content, Radical Scavenging Activity and Properties of Tablets Prepared from Noni Leaf and Fruit Extracts

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ABSTRACT

The purpose of this study was to evaluate the rutin content and radical scavenging activity of the leave and fruit extracts of *Morinda citrifolia* (Noni). The preparation and evaluation of tablets containing the Noni leaf and fruit extracts were also aimed. In this study, the leaves and fruits of Noni were prepared by maceration technique. Their rutin content and antioxidant activity were determined by using High Performance Liquid Chromatography (HPLC) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, respectively. The tablets containing theses extracts were prepared and their physical properties, including thickness and hardness, weight variation and disintegration time (DT) were evaluated. Additionally, the physicochemical stability was evaluated for three months period at 45 ^{o}C and relative humidity of 75 %. The results showed that Noni leave extract had similar rutin content and antioxidant activity compared to the fruit extract. The physical properties of tablets prepared from these extracts were met the requirements of USP XXX. Following stability test, rutin content and antioxidant activity of the extract and all tablet formulations were decreased.

Keywords: Morinda citrifolia L, *rutin content, radical scavenging activity, physical properties*

INTRODUCTION

Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against infections and degenerative diseases. Current research is now focused towards natural antioxidants originated from plants due to safe therapeutics. *Morinda citrifolia* L (Noni) (Family: Robiaceae), a medicinal plant commonly known in Thai as Yor, has

been reported to have broad therapeutic effects, including anti-inflammatory (McKoy et al. 2002), hypoglycemic (Nayak et al. 2011) and antioxidant (Wang et al. 2009) activities, in both clinical practice and laboratory animal models. In many countries, leaves and fruits of Noni are used as a treatment in traditional medicine. The leaves are mostly frequently used for external treatments such as rheumatic or swelling joints, broken bones and boil and burn, whereas the fruits used for internal treatment such as antiemetic agents, hypertension and diabetes (Pawlus and Kinghorn, 2007, Yang et al. 2011). It has been reported that the extract from Noni leaf and fruit exhibited a promising activity in DPPH scavenging and total phenolic content (Yang et al. 2011). Studies have shown Noni leaf and fruit contains several phenolic compounds including ursolic acid, kaempferol, quercetin and rutin. Rutin has been reported to have antioxidant, anti- diabetic and anti- inflammatory activity (Thani et al. 2010). Moreover, rutin has been used as a marker of antioxidant activity of Noni (Yang et al. 2011) and the differences of rutin content of Noni leaves and fruits have been not identified. Annually, the market sales of Noni products claimed to reach up to US $1.3 billion (Potterat and Hamburger, 2007). However, Noni products such as liquid dietary supplement or Tahitian Noni Juice are generally available in liquid dosage forms (Wang et al. 2009, Wang and Su, 2001). The liquid dosage form is inconvenient for the user, inaccurate amount of drug administration and unpleasant taste. Tablets are more preferable, stable and convenient alternative (Kucinskaite et al. 2007). Therefore, this investigation was aimed to determine the rutin content and radical scavenging activity of the leave and fruit extracts. The preparation and evaluation of tablets from the Noni leaf and fruit extracts were also included.

METHODOLOGY

Preparation of Noni leaf and fruit extracts

The leaves and fruits of Noni were collected from Phayao and Nakhon ratchasrima, Thailand, in August-October, 2013. The fresh leaves and fruits were washed and air dried. They were further dried at 50 ^{o}C using a hot-air oven (Memmert, Germany) for 48 hours. After grounding into powder, they were macerated using 95% ethanol. It was then filtered and concentrated using a rotary evaporator (Heidolph, Germany). Percentage yield of the crude extracts was calculated by the following equation:

% Yield = (weight of dried crude extract (g)/weight of dried noni (leaf/fruit) powder (g)) x 100 (1)

Determination of rutin content in extracts

The obtained extracts were analyzed for rutin, a marker, using High-Performance Liquid Chromatography (HPLC) (CBM-20Alite®, Shimadzu, Japan). Chromatography was performed on phenomenex luna C18 column (5 µm, 150x4.60 mm) with a mobile phase of 0.01M acetic acid: methanol: acetonitrile (60:20:20) and a flow rate of 1.0 mL/min. The volume injected is 20 µL with detection at 254 nm. The ruin content was calculated based on peak area of HPLC chromatogram.

Determination of antioxidant activity of the extracts

The antioxidant activity was determined using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay (Sigma Aldrich Chemicals, MO, USA) (Ghasemzadeh et al. 2011). Briefly, one mL of solution of Noni extracts was mixed with 9 mL of 0.2 mM DPPH in test tube. After incubation at RT for 30 min, the optical density at 515 nm (OD_{515}) of the mixture was determined by using UV-visible spectrophotometer (Jasco®, Japan). Ascorbic acid was used as positive control. Experimental was repeated three times. The ability to scavenge the DPPH radical was calculated by the following equation:

$$\% \text{ Radical scavenging} = (1-(\text{sample } OD_{515}/\text{blank } OD_{515})) \times 100 \quad (2)$$

Where the sample OD_{515} is the absorbance of the test sample and the blank OD_{515} is the absorbance of the blank (without test sample) at 515 nm.

Preparation and evaluation of Noni tablets

The tablets of Noni leaf and fruit extracts were prepared by wet granulation method. Two types of disintegrants including corn starch (CS) and sodium starch glycolate (SSG) were used. The ingredients and formulation code of Noni tablets are given in Table 1. Tablets were compressed by a hydraulic press (PerkinElmer, IL, USA) using a 10 mm in diameter round flat-faced punch at compaction force 2 kN. The prepared Noni tablets were further assessed for thickness and hardness (Erweka, Germany). Weight variation and disintegration time (DT, Erweka, Germany) based on the requirements of the USP XXX (2007).

Table 1. Ingredients and formulation code of Noni tablets prepared.

Ingredients	Functions	Amount (mg/tablet)					
		Rx-1	**Rx-2**	**Rx-3**	**Rx-4**	**Rx-5**	**Rx-6**
Leave extract	Active ingredient	8.0	-	-	8.0	-	-
Fruits extract	Active ingredient	-	16.0	-	-	16.0	-
PVP K30[a]	Binder	38.0 mg/tab					
Corn starch (CS)[b]	Disintegrant	83.0	83.0	83.0	-	-	-
Sodium starch glycolate (SSG)[b]	Disintegrant	-	-	-	83.0	83.0	83.0
Magnesium stearate	Lubricant	4.5 mg/tab					
Talcum	Lubricant	13.5 mg/tab					
Microcrystalline cellulose qs to	Diluent	500.0 mg/tab					

Note: [a] Binder was prepared as 10% w/w PVP K30, [b] Disintegrant was divided into 2 parts and added intra- and extra-granularly, [c] Ingredients were shown in amount of mg/ tablet

Stability test

The physicochemical stability was evaluated for three months period at 45 ^{o}C and relative humidity of 75%. Physical properties, rutin content and antioxidant activity of Noni tablets prepared were evaluated at day 0 and 90. For statistical analysis, the instrumental values were expressed as mean ± SD and were evaluated by oneway ANOVA with post hoc LSD test. All of these data, *p*-value of less than 0.05 was considered statistically significant difference.

Determination of rutin content in Noni tablets

For determination the rutin content in Noni tablets, the tablets prepared were triturated and then extracted by using methanol. The extract was filtered and 20 µL of filtrate was analyzed using HPLC as described above.

Determination of antioxidant activity of Noni tablets

For determination antioxidant activity of Noni tablets, the tablets prepared were triturated and then extracted by using ethanol. The extract was filtered and 1 mL of filtrate was assayed by DPPH method as described above.

RESULTS AND DISCUSSION

Rutin content and antioxidant activity of the extracts

The Noni leaf and fruit extract were viscous greenish-brown and viscous yellowish-brown gum, respectively. Percentage yield of Noni leaf and fruit extract obtained were approximately 30% and 18%, respectively (Table 2). Using HPLC technique, the rutin peaks were well-resolved under isocratic elution with retention times of 3.3 min and the rutin content in Noni leaf and fruit extracts were 0.54 and 0.42 % (w/w), respectively. The Noni leaf extract (IC_{50} = 1.35±0.02 mg/mL) was found to have similar antioxidant activity with the fruit extract (IC_{50} = 1.52 ±0.04 mg/mL). This is in disagreement with Yang et al. (2011) work in which the antioxidant activity of Noni leaf extract was greater than the fruit extract.

Table 2. The rutin content and antioxidant activity of leaf and fruit extract.

Sample	Yield (%)	Rutin content (% w/w)	DPPH radical scavenging activity (IC_{50})
Leave extract	29.61	0.54 ± 0.17	1.35 ± 0.02 mg/mL
Fruit extract	17.93	0.42 ± 0.09	1.52 ± 0.04 mg/mL
Ascorbic acid		-	9.82 ± 0.09 µg/mL

Physicochemical properties of Noni tablets

Based on the color of extracts, the tablets prepared from the leaves extract was light green in color, while the tablets prepared from the fruit extract was light brown in color (Figure 1). The tablets prepared without the extract was white in color. All prepared tablets had a smooth shiny surface with a round shape 10.00 ± 0.43 mm in diameter and an approximately 3.08 ± 0.08 mm thick.

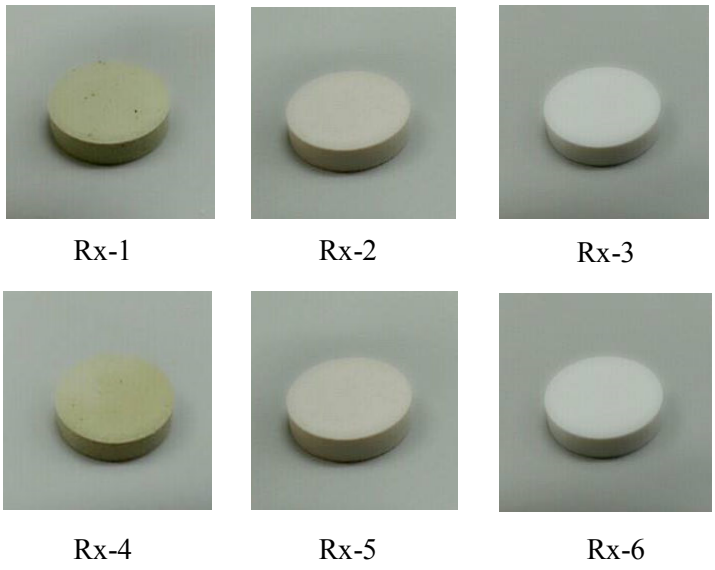


Fig. 1. The physical appearance of tablets prepared from various formulation.

The physical properties of all of tablet formulations are given in Table 3. It was observed that the average thickness of the tablets also ranged from 2.98-3.23 nm. The hardness was in the range of 8.76-14.22 kg in all formulations, which falls above the limit of not less than 6 kg indicating good mechanical strength for packing process and transportation of the finished product requirement. The weight of all formulations was approximately 500 mg with compression at the specified weight. The maximum of weight variation of the tablet was ± 4.00%, which falls within the acceptable weight variation range of ± 5.00%, thus all of tablet formulations passed the weight variation test. According to the requirement of USP XXX, tablets should disintegrate within 15 min. It was observed that all formulations disintegrated within less than 6 min and the tablets prepared by using SSG presented slightly shorter disintegration time.

Table 3. Physical properties of tablets containing Noni leaf and fruit extracts before stability test.

Formulation	Physical properties				
	Hardness (kg)	Thickness (mm)	Weight variation (mg)	Weight variation (%)	Disintegration time (min)
Rx-1	8.76±0.32	3.15±0.03	501.80±2.30	4.00	4.54±0.31
Rx-2	10.08±0.41	3.03±0.04	502.30±2.30	3.69	4.07±0.25
Rx-3	14.22±0.57	3.23±0.06	496.90±7.10	3.46	5.15±0.07
Rx-4	11.02±0.81	3.03±0.03	508.80±6.10	1.90	3.33±0.15
Rx-5	12.66±0.44	2.98±0.02	501.00±3.20	1.60	3.24±0.17
Rx-6	13.74±0.76	3.08±0.05	501.70±2.20	3.40	3.51±0.26

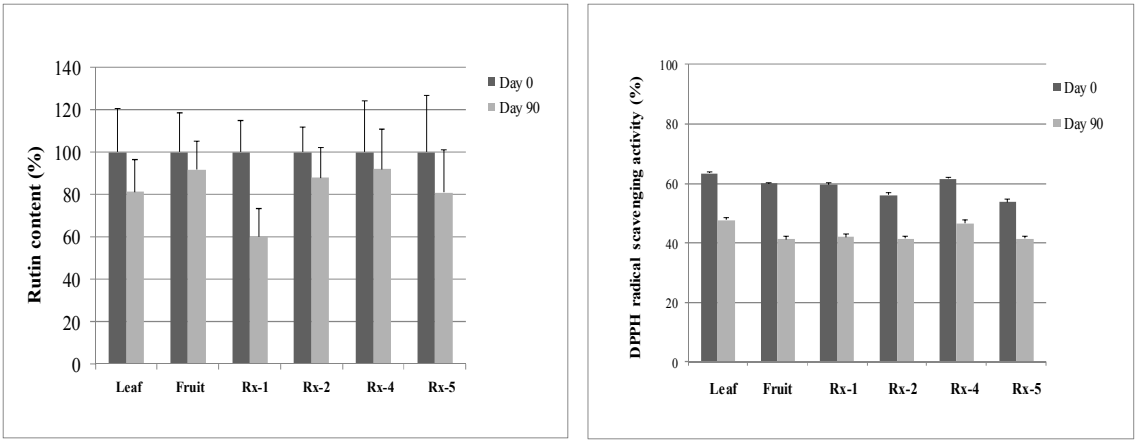
***** Note: The requirement of USP standard: Disintegration time (DT) < 15 min

Table 4. Physical properties of tablets containing Noni leaf and fruit extracts following stability test.

Formulation	Physical properties				
	Hardness (kg)	Thickness (mm)	Weight variation (mg)	Weight variation (%)	Disintegration time (min)
Rx-1	6.47±0.36	3.07±0.05	502.40±8.51	5.40	0.26±0.03
Rx-2	7.34±0.56	3.11±0.05	506.90±0.43	2.73	2.31±0.11
Rx-3	12.13±0.61	3.01±0.03	501.00±0.60	1.54	0.19±0.04
Rx-4	9.45±1.81	3.21±0.03	511.00±0.40	1.71	4.01±0.04
Rx-5	11.42±0.04	3.21±0.03	503.80±0.30	1.71	3.62±0.04
Rx-6	9.18±1.78	3.38±0.05	508.80±0.47	3.16	3.96±0.03

After stability test, the physical properties of all of tablet formulations are presented in Table 4. Compared to the data of day-0 tablets, the hardness of all tablet formulations was decreased whereas the thickness and weight variation of the tablets were increased. However, the variations were not alarming and remained within the acceptable range except Rx-1 with the maximum of weight variation of the tablet was ± 5.40% (Table 4). The hardness of tablets prepared was reduced what resulted in faster disintegration time except tablets prepared using SSG. Overall, Rx-5 showed better physical properties compared with the other formulations. The rutin content in the leaf, fruit extracts and all Noni tablet formulations were decreased following stability as shown in Figure 2a. However, the decrease in rutin content of the extracts and all tablet formulations were not significant. The DPPH scavenging activity of Noni leaf and fruit

extract in crude extract and in tablet formulations were decreased significantly ($p < 0.05$) (Figure 2b).



a b

Fig. 2. The rutin content (%) (a) and the DPPH radical scavenging activity (%) (b) of Noni leaf, fruit extract and all Noni tablet formulations (when analyzed at a concentration of the extract at 2 mg/mL) following stability test.

CONCLUSIONS

The present study demonstrated that Noni leave extract had similar rutin content and antioxidant activity compared to the fruit extract. The tablets containing Noni leaf and fruit extract were successfully prepared with the satisfied physical properties base on the requirement of USP XXX. Rutin content and antioxidant activity of the extract and all tablet formulations were decreased after stability test.

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REFERENCES

McKoy, M.L.G., Thomas, E.A. and Simon O.R. (2002). Preliminary Investigation of the Anti-inflammatory Properties of an Aqueous Extract from *Morinda citrifolia* (Noni). *Proc. West. Pharmacol. Soc*, 45, 76–78.

Nayak, B.S., Marshall, J.R., Isitor, G.A. and Adogwa, A. (2011). Hypoglycemic and Hepatoprotective Activity of Fermented Fruit Juice of *Morinda citrifolia* (Noni) in Diabetic Rats. *Evid. Based Complement. Alternat. Med*, 1-5.

Wang, M.Y., Peng, L., Hoper V.W., Deng, S., Anderson, G. and West, B.J. (2009). Noni Juice Improves Serum Lipid Profiles and Other Risk Markers in Cigarette Smokers. *Scientific World J*, 1-8.

Pawlus A.D. and Kinghorn D. A. (2007). Review of the ethnobotany, chemistry, biological activity and safety of the botanical dietary supplement *Morinda citrifolia* (noni). *J. Pharm. Pharmacol*, 59(12), 1587-1609.

Yang, J, Gadi, R. and Thomson, T. (2011). Antioxidant capacity, total phenols, and ascorbic acid content of noni (*Morinda citrifolia*) fruits and leaves at various storage of maturity. *Micronesica,* 41(2), 167-176.

Thani, W., Vallisuta, O., Siripong, P. and Ruangwises, N. (2010). Anti-proliferative and antioxidative activites of Thai noni/Yor (*Morinda citrifolia* Linn) leaf extract. *Southeast Asian J. Trop. Med. Public Health,* 41(2), 482-489.

Potterat, O. and Hamburger, M. (2007). *Morinda citrifolia* (noni) fruit–phytochemistry, pharmacology, safety. *Planta Med*, 73(3), 191–199

Wang, M.Y. and Su, C. (2001). Cancer Preventive Effect of *Morinda citrifolia* (Noni). *Ann. N. Y. Acad. Sci,* 952, 161-168.

Kucinskaite, A., Sawicki, W., Briedis, V. and Szitowska, M. (2007). Fast Disintegrating Tablets Containing *Rhodiola rosea* L. Extract. *Acta Pol. Pharm*, 64(1), 63-67.

Ghasemzadeh, A., Jaafar, H. Z. E. and Rahmat, A. (2011). Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (*Zingiber officinale* Roscoe) extracts. *J. Med. Plants Res*, 5(7), 1147-1154.

United States Pharmacopoeia XXX. The USP Convention. 2007.