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RESEARCH ARTICLE

Phytochemical investigation of noni (*Morinda citrifolia* L.) leaves extract applicated for sunscreen product

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Abstract

Morinda citrifolia L. (noni) is an annual plant, distributed in the tropical parts of the world. Chemical constituents such as flavonoids, alkaloids, terpenoids, sterols, and minerals have been isolated from this plant. The pharmacological properties, based on scientific research, claimed as antibacterial, antiulcerogenic, anti-inflammatory, antioxidant, and especially for anti melanogenesis therapy. Iridoid, a flavonoid compound, is the main component found in the noni leaves extract. The quantitative analysis was performed by TLC densitometry using CAMAG TLC Scanner 3 and WinCATS software version 1.3.4 (Switzerland). Quantification was performed using calibration curves (peak area of chromatogram versus the mass of standard applied in the form of the band) for the individual standard in triplicate. The results showed that the noni leaves extract possessed flavonoid and steroid content with the concentration of 2.82% and 0.14%, respectively. Therefore, the noni leaves extract is potentially used in sunscreen, based on anti-melanogenesis activity.

Keywords: Anti-melanogenesis, flavonoid, noni

INTRODUCTION

Morinda citrifolia L. (Rubiaceae), commonly known as noni, is indigenous to tropical regions, such as French Polynesia, Tonga, Hawaii, Australia, and other islands of the Pacific area [1]. The other names of *Morinda citrifolia* L. include *M. bracteata* Roxb; *M. litoralis* Blanco, Indian mulberry, Bengkudu, and Mengkudu (Malay). It has been reported to have a broad range of therapeutic effects for subjects with tumor, cancer, infections, inflammation, arthritis, diabetes, asthma, hypertension and pain. The Polynesians utilized the whole *Morinda citrifolia* L. plant in their traditional remedies and as a dye for some traditional clothing. The roots, stems, bark, leaves, flowers, and fruits of the *Morinda citrifolia* L. plant are all involved in various combinations and recorded herbal medicines [2].

The major components have been identified in the noni plant such as scopoletin, octanoic acid, potassium, vitamin C, terpenoids, alkaloids, anthraquinones [3] and iridoids, such as asperuloside, asperulosidic acid, deacetylasperuloside, citrifolinin, citrifoside, and dehydroepoxymethoxygaertneroside [4]. The iridoid, which belongs to flavonoid compound, was mainly found in the Noni leaves. These constituents may play a major role as an antioxidant [5]. The negligible antioxidant activities of *Morinda citrifolia* leaves were reported by Zin *et al.* [6] using thiobarbituric acid test (TBA) and ferric thiocyanate method (FTC).

Melanogenesis is a multistage process involving melanin synthesis, melanin transport, and melanosome release. Tyrosine is one of the key enzymes, which hydroxylates tyrosine to dihydroxyphenylalanin (DOPA) and oxidizes DOPA to dopaquinone. The dopaquinone plays a role in the melanin biosynthetic pathway. Abnormal deposition of melanin pigment causes hyperpigmentary disorders, such as melasma, freckles, and spots. Tyrosinase inhibitor is one of the candidates for reduction of melanogenes [7]. The study of anti-melanogenesis activity was carried out using an *in vitro* tyrosinase inhibition assay with 50% ethanol extracts of fruit, leaves, and seeds. The results showed that noni leaves extract has anti-melanogenesis activity [8].

The tyrosinase inhibitory activity of noni leaves extract was not as potent as skin whitening agents. However, in the B_{16} melanoma cells culture system, it significantly inhibited melanogenesis. Thus, noni may be useful as an anti-photoaging cosmetic ingredient which prevents and treats the pigmented spot and wrinkle skin [9]. Therefore, this compound was potential to be formulated into sunscreen products.

EXPERIMENTAL

Materials

Plant materials: The dried extract of Noni leaves (*Morinda citrifolia* L.) was obtained from Borobudur Extraction Center, Semarang, Central Java, Indonesia.
Chemicals: The analytical grade was used for all chemicals and solvents. Mg powder (China), HCl (China), amyl alcohol (China), Dragendroff's reagent (China), ferric chloride (China), sodium hydroxide (China), ether (China), anhydrate acetic acid (China), sulfate acid (China), and ready-made silica gel TLC plates (Merck, Germany) were used.

Phytochemical screening

Test for flavonoids: The extract was dissolved in alcohol, to which few magnesium turnings will be added followed by dropwise of concentrated HCl. After shaking well the mixture, the appearance of red color in the amyl alcohol layer suggested the presence of flavonoids [10].
Test for alkaloids: The extract which was diluted in hydrochloric acid was filtered. The filtrate was then treated with various alkaloidal reagents [10]. Dragendroff's test: The filtrate was added by the Dragendroff reagent and the appearance of reddish brown precipitate indicated the presence of alkaloids compound [10].

Test for saponins: The 1 ml of the extract was diluted to 20 ml of distilled water and shaken well in a test tube. The formation of foam in the upper part of test tube indicated the saponins compound [10].
Test for tannins: The extract was treated with a neutral ferric chloride solution, the appearance of greenish black color indicated the tannins compound [10].
Test for steroids: The extract was added with ether, anhydrate acetic acid, and sulfate acid. The appearance of red color indicated the presence of steroids [10].

Quantitative analysis

Thin layer chromatography was performed on 10 × 20 cm TLC silica gel 60 F_{254} plates. Flavonoids and steroid tests were carried out using rutin (10 mg) and stigmasterol (10 mg) as the standard, respectively. A mixture of ethyl acetate : formic acid : acetic acid : water in the volume ratio of 100 : 11 : 11 : 27 was used as the mobile phase of flavonoids. A mixture of hexane : ethyl acetate (4 :1) was used as the mobile phase of steroids. All plates were visualized directly after drying and with the help of UV at 366 nm in UV TLC viewer. The identification and quantification were performed by TLC densitometry using CAMAG TLC Scanner 3 and WinCATS software version 1.3.4 (Switzerland). Quantification was performed using the calibration curves (peak area of chromatogram versus the mass of standard applied in the form of the band) for the individual standard in triplicate [11].

RESULTS AND DISCUSSION

Phytochemical screening of secondary metabolites in *Morinda citrifolia* L. leaves were conducted using color tests. These tests were used to determine the class of secondary metabolites, such as alkaloids, flavonoids, polyphenols, terpenoids/steroid, saponins and tannins of the leaves [12]. The active compound from noni leaves extract were flavonoids, alkaloids, saponins, tannins, and steroids, which were indicated as symbol (+) in Table 1.

TLC is suitable for initial orientation analysis of plant extracts. Flavonoids have two benzene rings separated by propane and is a derivative of flavone. In general, they are water-soluble compounds. The conjugated compound has been dominantly found when they have a brighter color. In the plants, flavonoid has been commonly found in the form of glycosides [13]. The flavonoids are classified by the addition of oxygen, a heterocyclic ring, and hydroxyl groups. These groups include catechins, leucoanthocyanidin, flavanones, flavanonol, flavones, anthocyanidins, flavonols, chalcone, aurone, and isoflavones. With regard to detection, flavonoids on TLC plates produce a yellow-brown spot on a white background when reacted with iodine vapor [14].

Flavonoids may appear as dark spots on a green background fluorescent when observed under UV light at 254 nm of UV-plates containing a fluorescent indicator (such as silica gel F_{254}). Under 365 nm UV light, the observed spot colors would depend on the structure of flavonoids; it can be yellow, green or blue fluorescent. It would be clearer and more intense after being sprayed with the reagent [15].

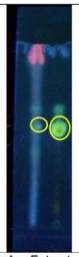
Noni leaves extract has flavonoid compound which gave a sharp and well-defined band with R_f of 0.50 (Table 2). The spot color was yellow, which was the same color as rutin. Rutin was used as the standard because it belongs to flavonoid glycosides. This compound has been observed for the most of plants, especially in leaves part.

Steroids play important functional roles in plants. Phytosterols are integral components of the plant cell membrane lipid bilayer that control the membrane fluidity and permeability [16]. On the other hands, stigmasterol could be found as a large part of phytosterol. This compound is an unsaturated plant sterol occurring in the plant fats or oils of many plants and medicinal herbs [17]. The spots of the stigmasterol and noni leave extract have the same color (white) and similar Rf values to each other, where the stigmasterol and noni leaves extract on TLC plates were both detected about 0.50. This result confirmed that the steroid was one of the components existed in the noni leaves extract.

Table 1 Phytochemical screening results.

Test	Reagent	Positive indicator [12]	Observation	
			Visual	Result
Flavonoid	Mg powder + HCl + amyl alkohol	Red color at amyl alcohol's layer was observed.		(+) Red color at amyl alcohol's layer
Alkaloid	HCl + Dragendorff reagent	Red precipitate was formed.		(+) Red precipitate
Saponin	HCl 2N	After shaking well, one centimeter layer of foam was observed.		(-)
Tannin	$FeCl_3$ 1%	Blackish green color was observed.		(+) Blackish green
Steroid/ Terpenoid	Ether + anhydrate acetic acid + H_2SO_4	Red /violet/green color was observed.		(+) Red color

Table 2 TLC analysis.

Test	Observation	
	Visual	Under UV (366 nm)
Flavonoid		
Description of Rf and color		A = Extract B = Standard A = 0,50 B = 0,51 A = Yellow B = Yellow

Test	Observation	
	Visual	Under UV (366 nm)
Flavonoid		
Description of Rf and color		A = Extract B = Standard A = 0,50 B = 0,50 A = White B = White

Quantitative measurements on TLC are carried out usually by TLC densitometry. Densitometry is the quantitative measurement of the optical density of a light-sensitive substance, which can be used to determine a spot on a thin-layer chromatogram [18]. The quantification of steroid was carried out using TLC densitometry at 366 nm. A precise and accurate quantification of this compound has

been achieved with stigmasterol standard at concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 µg/µL (Table 3).

Table 3 Standard calibration of stigmasterol.

Standard : Stigmasterol 10 mg/mL		
No	Standard (µg/µL)	Area
1	0.1	6287.99
2	0.2	7705.83
3	0.3	9036.65
4	0.4	10800.08
5	0.5	12166.35
6	0.6	13378.41

The standard curve of stigmasterol is shown in Fig. 1. The determination of steroid in the noni leaves extract was made by plotting the area of stigmasterol obtained from analyzed samples. Table 4 shows that each area of the analyzed samples was in the range of the area of stigmasterol standard. Based on the plot, the quantity of steroid in noni leaves extract was determined to be 0.14%.

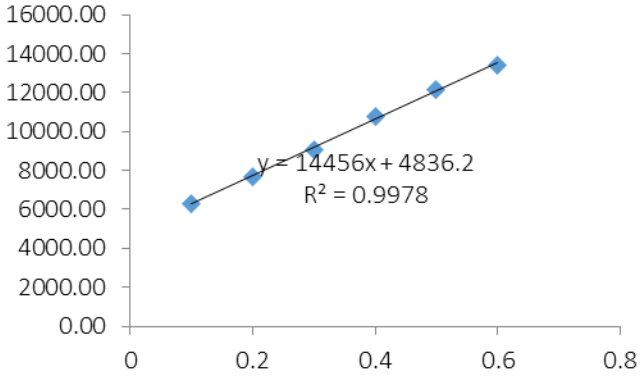


Fig. 1 A standard curve of stigmasterol.

Table 4 Quantitative test of steroids.

Sample	Weight (mg)	Area	Concentration (%)
Extract 1	100.5	6902.71	0.14
Extract 2	100.5	6908.16	0.14
Extract 3	100.5	6935.15	0.14

The determination of flavonoid content was carried out by UV-vis spectrophotometer at a wavelength of 366 nm. The aluminum chloride ($AlCl_3$) was the key reagent to measure the flavonoid. This substance can make a complex binding, which could be indicated with the appearance of violet color. As shown in Table 5 and Fig. 2, rutin was used as a standard and the calibration curve could be derived following the equation of $y = 0.007x + 0.0555$ R^2 0.9983, where y is an absorbance at 366 nm and x is flavonoid content in the leaves extracts of *Morinda citrifolia* L. with a concentration in mg/g. The flavonoid content was approximately 2.82% in the ethanolic extracts (Table 6).

Table 5 Standard calibration of rutin.

Standard : Rutin 10 mg/5mL		
Volume test (mL)	Concentration	Abs + $AlCl_3$
1	2	0.094
5	10	0.126
10	20	0.198
40	80	0.580
50	100	0.736
100	200	1.470

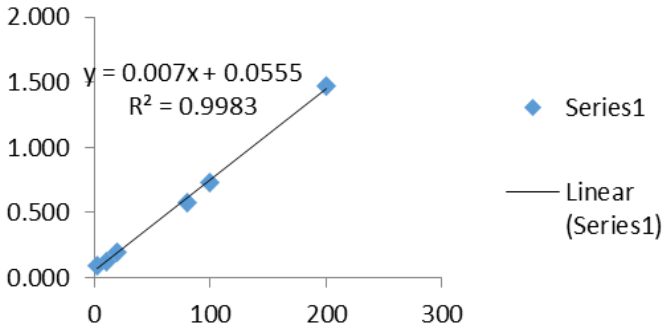


Fig. 2 A standard curve of rutin.

Flavonoid seems to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. Flavonoid is present in plant extracts that have been used for medicinal purposes, such as sunscreen and antioxidant [19].

Table 6 Quantitative test of flavonoids

Sample	Weight (mg)	Area	Concentration (%)
Extract 1	100.5	0.254	2.82
Extract 2	100.5	0.254	2.82
Extract 3	100.5	0.254	2.82

CONCLUSION

The current study revealed the presence of various bioactive constituents in the leaves extract of *M. citrifolia*. The objective of this study was to provide the qualitative and quantitative information about steroids and flavonoids in ethanolic extract of *M. citrifolia*. Further work of this study is to correlate relationship of these active constituents for possible biological activities and evaluate *M. citrifolia* as a potential source of natural bioactive chemicals for sunscreen product. Further studies are still ongoing concerning this plant in order to study the structure of bioactive compounds by various techniques such as high-performance liquid chromatography (HPLC), Fourier transform infrared (FTIR) spectroscopy and nuclear magnetic resonance (NMR). The preliminary phytochemical investigation will further help in isolation and formulation into sunscreen product.

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