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Coumarin Derivative from the Unripe Fruit of Mengkudu (*Morinda citrifolia* Linn) and Cytotoxic Activity

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Abstract

A coumarin derivative namely scopoletin (7-hydroxy-6-methoxychromen-2-one) had been isolated from the methanol extract of unripe noni fruits (*Morinda citrifolia* Linn.). The extraction was conducted by maceration, continuing with separation and purification using several techniques of chromatography. The yellowish isolated compound showed a melting point at 200 – 202 °C. The compound structure was analyzed based on spectral data ^{1}H-NMR, ^{13}C-NMR, and NOE 1D. The inhibition of methanol extract of noni fruits and scopoletin against murine leukemia P388 cells using MTT assay showed IC_{50} were > 100 µg/mL and 65.69 µg/mL respectively. These showed that scopoletin was more cytotoxic than methanol extract but weaker than artonin E as a positive control IC_{50} 0.48 µg/mL).

Keywords: Noni (Morinda citrifolia Linn.), coumarin, scopoletin, cytotoxicity, murine leukemia P388 cells

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Abstrak (Indonesia)

Senyawa turunan kumarin yaitu skopoletin telah berhasil diisolasi dari ekstrak metanol buah mengkudu (*Morinda citrifolia* Linn.) mentah. Proses ekstraksi dilakukan secara maserasi, dilanjutkan dengan pemisahan dan pemurnian menggunakan beberapa teknik kromatografi. Senyawa berwarna kuning hasil isolasi memberikan titik leleh pada 200 – 202°C. Struktur senyawa ditentukan berdasarkan data spektrum ^{1}H-NMR, ^{13}C-NMR, dan NOE 1D. Penghambatan ekstrak metanol buah mengkudu dan skopoletin terhadap sel murin leukemia P388 menggunakan metode MTT memberikan nilai IC_{50} berturut-turut sebesar > 100 µg/mL and 65,69 µg/mL. Hasil ini menunjukkan bahwa skopoletin lebih sitotoksik dibandingkan ekstrak metanol namun lebih lemah dari kontrol positif (artonin E dengan IC_{50} 0.48 µg/mL).

Kata kunci: Mengkudu (Morinda citrifolia Linn.), kumarin, scopoletin, sitotoksik, sel murine leukemia P388

INTRODUCTION

Secondary metabolites are a source of medicinal compounds both used in traditional and modern medicine. Plants such as mengkudu are producers of various secondary metabolites that can be utilized for this purpose. Mengkudu is the Indonesian local name for the noni plant (*Morinda citrifolia*). It is a perennial plant grown in tropical and subtropical regions in South Pacific islands and Southeast Asia [1, 2]. This plant has been widely used as food, dye, and herbal remedies to cure several diseases [3]. Almost all parts of this plant, such as fruit, flowers, leaves, bark, and roots, have been used as a traditional medicine to treat various diseases such as diabetes, hypertension, dysentery, infections, muscle aches, treating swollen spleen, liver diseases, cough inflammation, and anticancer [3-5]. Indonesian people, especially Bugis, used this plant's fruits for bad breath or body odor remover and to treat hypertension [6].

Various phytochemicals have been identified from all parts of mengkudu plant, including phenolic compounds such as coumarins, flavonoids, lignans, and anthraquinones, as well as non-phenolic compounds ie. polysaccharides, iridoids, triterpenoids and steroids [7-10]. Especially from the fruits, the main compound including damnachantal (an anthraquinone), scopoletin (a coumarin), rutin (a flavonoid), asperuloside (an iridoid), and ursolic acid (a triterpenoid) [7]. Previous researchers have isolated antioxidant lignans from the fruit as well as

anthraquinones that are potent as quinone reductase-inducing activity in Hepa lclc7 cells from the root and fruits of this plant. Quinone reductase led to decreased harmful radicals, hence it's exerts cancer chemoprotective properties [11]. This bioactivity showed the potential of these plant fruits as anticancer. Another report showed the safety of using noni fruit juice as an anticancer drug [12]. Previously, we have reported a hexadecanoic acid and an alkenyl glycoside from the fruit of mengkudu [13]. As a part of our research to find the source of drugs for anticancer agents, hence this paper describes the isolation of coumarin from the unripe fruits and cytotoxic activity from the crude extract and isolated compound against murine leukemia P388 cells as a preliminary assay for an anticancer agent.

MATERIALS AND METHOD

General experimental procedures

Chromatography techniques using silica gel 60 (Merck) for Vacuum Liquid Chromatography (VLC), silica gel 60 PF_{254} (Merck) for centrifugal chromatography, and silica gel 60 GF 254 precoated on aluminum plates (Merck) for TLC analysis. TLC visualization using UV lamp CAMAG 254 and spraying with CeSO4 1,5% in H_2SO_4 2N then heated. The melting point was measured by Fisher Johns melting point apparatus. NMR spectra were recorded by Agilent DD2 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C) using residual solvent as the reference standard. ELISA reader was used for the MTT assay.

Plant material

The unripe fruits of *M. citrifolia* were collected from Lunjuk Jaya, Palembang, South Sumatera, and identified at the Biology Department, Faculty of Mathematics and Natural Science, Sriwijaya University, South Sumatera, Indonesia.

Extraction and isolation

A total of 850 g of dry noni fruit powder was extracted by maceration (1.5 L methanol solvent for 1 x 24 hours) and repeated up to 3 times. The filtered extract was then evaporated to obtain 124 g of crude methanol extract. Part of the crude extract (69.5 g) was dissolved using acetone. The dissolution process contained soluble and insoluble parts. The soluble part obtained (8.6 g) was separated using VLC (silica gel G 60; 100% *n*-hexane eluent, *n*-hexane: ethyl acetate (8:2, 6:4, 1:1, 3:7), 100% ethyl acetate, ethyl acetate: methanol (9:1, 8:2) and 100% methanol) with an eluent volume of 100 mL/elution. This separation produces 8 fractions (A–H).

The D fraction (279.6 mg) was further separated by centrifugal chromatography (1 mm plates; eluent *n*-hexane: chloroform (3:7, 2:8, 1:9, 0:10), chloroform: methanol (98:2) and methanol 100%) with eluent volume of 20 mL/elution to obtained 7 fractions. The fraction of D3 (71,0 mg) continued separation by the same method using *n*-hexane: acetone (8:2, 7:3, 6:4) and methanol 100% as the eluent to produce 3 fractions. Centrifugal chromatography on D32 fraction (29.6 mg) with the same method to afforded compound (**1**) 24.1 mg).

Cytotoxic Assay

The cytotoxicity of methanol crude extract and isolated compound against murine leukemia cells P388 was determined by the MTT test method [14]. P388 murine leukemia cells were suspended in RPMI media containing FBS. The first day of cell culture was inoculated into 96-well plates and incubated in a CO_2 incubator (37 ^{o}C). On the second day, samples were added. As much as 1 mg for each sample was dissolved prior in 1 mL of DMSO (stock solution) then variations of concentration were made (100; 30; 10; 3; 1; 0.3; 0.1) µg/mL using PBS buffer solution with a pH of 7.30–7.65. Each sample with various concentrations was added in triplicate to each well-containing cell culture and then stored in a CO_2 incubator (37 °C) for 48 hours. All stages of work are carried out aseptically in laminar airflow.

The positive control consisted of cell culture and DMSO solvent, while the standard compound artonin E (IC_{50} 0.7 µg/mL) was used to compare. After 48 hours, 0.5 mg/mL MTT reagent was added to each well, then shaken and stored again in a CO_2 incubator (37 ^{o}C) for 4 hours. The MTT reduction reaction was stopped by adding an SDS reagent. Cell incubation was continued for 24 hours in the dark at room temperature. The absorbance of each well was read at λ 550 nm using a microplate reader. The cytotoxic effect of the test compound was determined based on the IC_{50} value obtained by interpolating the 50% absorbance line of the positive blank on the semi logarithmic graph which states the relationship between absorbance and variation in the concentration of the test sample (µg/mL).

RESULTS AND DISCUSSION

The isolated compound was a yellowish solid with m.p 200-202 °C. On TLC analysis, the isolated compound showed only one spot in *n*-hexane: ethyl acetate (7:3), *n*-hexane: chloroform (1:9), and chloroform: methanol (9:1) with the Rf value was 0.17; 0.24 and 0.83 respectively. (**Figure** 1) which indicated that the isolated compound was pure.

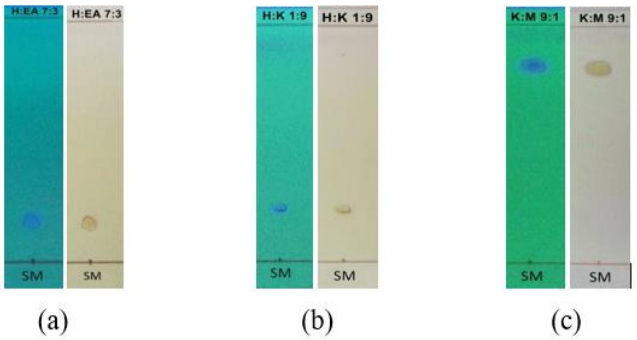

Figure 1. The TLC of the isolated compound using various eluent; a) *n*-hexane:ethyl acetate (7:3), (b) *n*-hexane:chloroform (1:9), and (c) chloroform:methanol (9:1)

The ^{1}H-NMR spectrum (500 MHz, acetone-d_6) (**Figure** 2) showed two singlet signals at δ_H 6.79 and δH 7.18 ppm each representing one proton. The two signals originate from aromatic protons coupled to each other through the 1,2,4,5-tetrasubstituted phenyl unit. The presence of vinylic unit was shown in the ^{1}H-NMR spectrum as 2 doublet signals at δ_H 6.17 ppm (1H, *J*= 9.5 Hz) and δ_H 7.83 ppm (1H, *J*= 9.5 Hz). The coupling constant of the two doublet signals indicates that the proton orientation of the vinylic unit was cis. A singlet signal for three protons at δ_H 3.90 ppm (3H, *s*) was characteristic of the presence of a methoxy unit, meanwhile, a broad singlet signal at δ_H 8.78 ppm (1H, *s*) indicated the presence of a hydroxyl group on the aromatic ring.

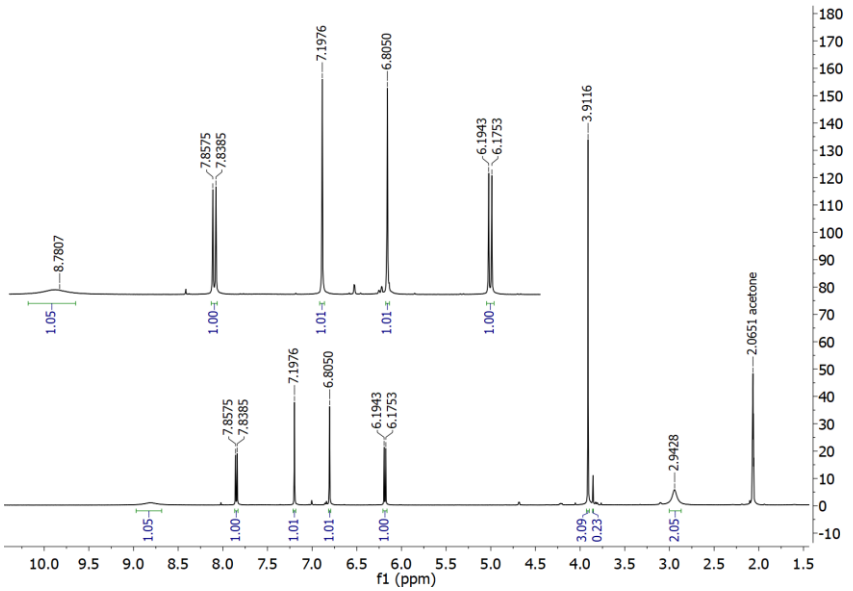

Figure 2. The ^{1}H-NMR spectrum of the isolated compound

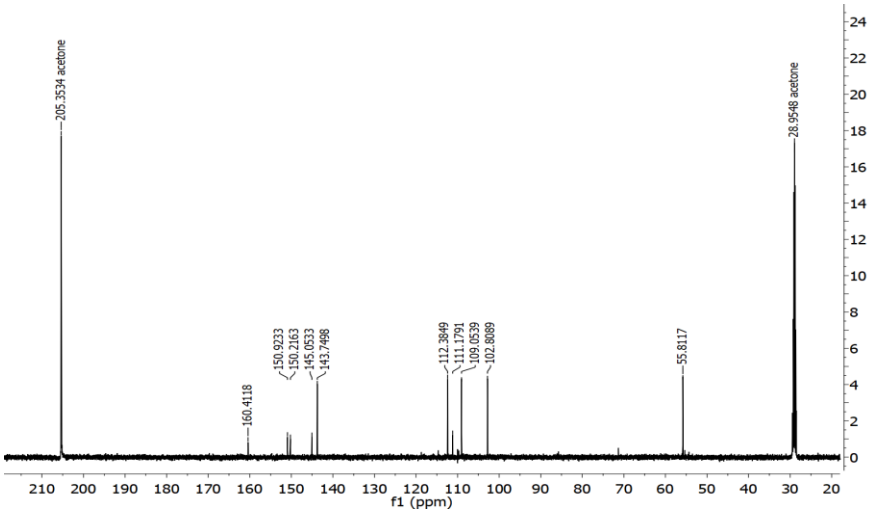

Figure 3. The ^{13}C-NMR spectrum of the isolated compound

The ^{13}C-NMR spectrum (125 MHz, acetone-d_6) (**Figure** 3) shows the presence of 10 signals for 10 carbon atoms. The signal consists of a signal for carbonyl ester typical at δ_C 161.3 ppm. Actually, the carbonyl chemical shift of carboxylic acid and its derivatives is 155-185 ppm [15]. The isolated compound's carbonyl signal was shielded, indicating the presence of a,b-unsaturated resonance toward the carbonyl group. Another 3 signals with weak intensity at δC 151.8; 151.1, and 146.0 ppm indicating the presence of three oxy-aryl carbons which appear as a distinctive signal in the δ_C 140–155 ppm region, a signal at δ_C 112.0 ppm representing the C-quaternary atom, and four other signals with moderate intensity at δC 144.7; 113.3; 110.0 and 103.7 ppm indicated the presence of 4 sp^2-methine carbons. The presence of a methoxy group was strengthened by the appearance of a carbon signal at δ_C 56.7 ppm.

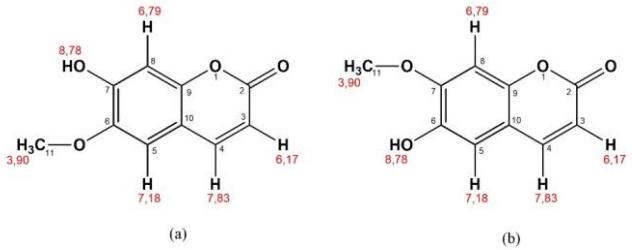

Figure 4. Possibility structures of the isolated compound

Based on ^{1}H-NMR and ^{13}C-NMR data, the compound was a coumarin derivative with a hydroxyl and a methoxy group with two possible structures (**Figure** 4). The proton signal at C-5 should be deshielding than the proton signal at C-8 caused by the conjugated effect from carbonyl in C-2. Confirmation of the methoxy and the hydroxyl group position was obtained from the 1D NOE experiment. Through the NOE spectrum (Nuclear Overhauser Effect) we can observe protons that are close together in space.

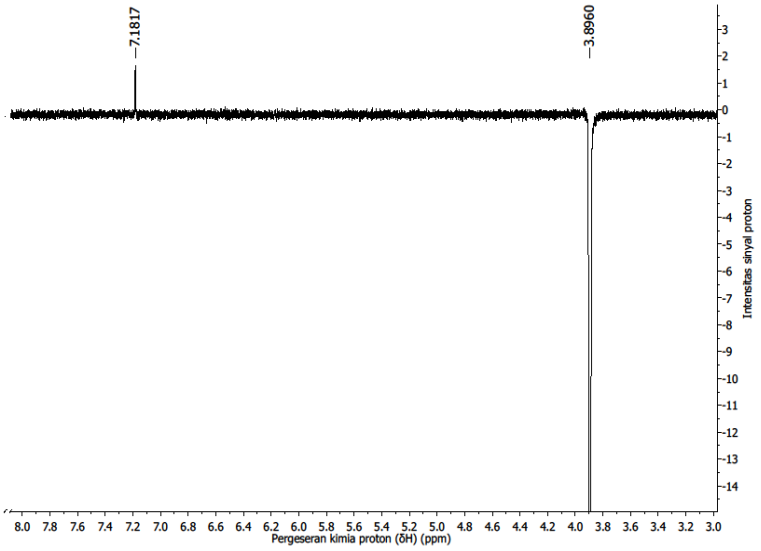

Figure 5. The 1D-NOE spectrum of the isolated compound

Table 1. NMR data of the isolated compound and the reference compound

Number	isolated compound (acetone-d_6)		Scopoletin* ($CDCl_3$) [16]	
	δ_C (ppm)	δ_H (ppm) (∑H; multiplicity; *J*)	δ_C (ppm)	δ_H (ppm) (∑H; multiplicity; *J*)
2	161.3	-	160.5	-
3	113.3	6.17 (1H; *d*; 9.5 Hz)	112.5	6.26 (1H; *d*; 9.4 Hz)
4	144.7	7.83 (1H, *d*; 9.5 Hz)	142.0	7.58 (1H, *d*; 9.4 Hz)
5	110.0	7.18 (1H, *s*)	107.5	6.89 (1H, *s*)
6	146.0	-	143.0	-
7	151.1	-	149.5	-
8	103.7	6.79 (1H, *s*)	102.0	6.82 (1H, *s*)
9	151.8	-	150.0	-
10	112.1	-	110.5	-
–OCH_3	56.7	3.90 (3H, *s*)	55.2	3.93 (3H, *s*)
–OH	-	8.78 (1H, *s*)	-	-

In the NOE experiment, irradiation of the methoxy proton at δ_H 3.90 ppm only left a proton signal at δ_H 7.18 ppm as shown in **Figure** 5. This proved that the methoxy group was close to the proton at 7.18 ppm not to the proton at 6.79 ppm, so the correct structure for the isolated compound was structure (a) in Figure 3 namely scopoletin. The assignment of protons and carbons signal of the isolated compound was compared with the scopoletin reported and showed high similarity [16] (Table 1).

The cytotoxicity assay against murine leukemia P388 cells-line was carried out on methanol extract of noni fruit and the isolated compound (scopoletin) (**Table** 2). This cell-line was used to screening candidate anticancer agents. The results showed the scopoletin (IC_{50} 65.69 µg/mL) was more cytotoxic than the methanol extract with IC_{50} > 100 µg/mL. These results were congruent with those that scopoletin was more cytotoxic than the crude extract against the melanoma cell line (WM115) and osteosarcoma cell line (Saos 2) with IC_{50} values of 5 and 10 µg/mL respectively [17]. Another report showed that scopoletin had a slight inhibitory effect against four human cancer cells KB, HeLa, MCF-7 and HepG2, as well as non-tumorigenic Vero cells, with IC_{50} > 100 µg/mL [18].

Table 2. Cytotoxicity of the sample against murine leukemia P-388 cells

Sample	IC_{50} (mg/mL)
methanol extract	>100
scopoletin	65.69
artonin E	0.48

CONCLUSION

In summary, a secondary metabolite was successfully isolated and characterized from the unripe fruit of mengkudu namely scopoletin (7-hydroxy-6-methoxychromen-2-one). The cytotoxic activity of the isolated compound was better than methanol crude extract against murine leukemia P388 cells.

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REFERENCES

[1] R. Wang, L. Wang, L, Zhang, S, Wan., C. Li., and S. Liu, "Solvents effect on phenolics, iridoids, antioxidant activity, antibacterial activity, and pancreatic lipase inhibition activity of noni (*Morinda citrifolia* L.) Fruit Extract," *Food Chemistry*, vol. 377, p. 131989, 2022.

[2] H. Sina, G. Dramane, P. Tchekounou, M.F. Assogba, K. Chabi-Sika, B. Boya, A. Socohou, A. Adjanohoun, L. Baba-Mouss, "Phytochemical composition and in vitro biological activities of *morinda citrifolia* fruit juice," *Saudi Journal of Biological Sciences*, vol 28, pp. 1331-1335, 2021.

[3] G.C.L. Ee, Y.P Wen, M.A. Sukari, R. Go, and H.L. Lee, "A New anthraquinone from morinda citrifolia roots," *Natural Product Research*, vol. 23, no. 14, pp. 1322–1329, 2009.

[4] M. Wang, H. Kikuzaki, Y. Jin, N. Nakatani, N. Zhu, K. Csiszar, C. Boyd, R.T. Rosen, G. Ghai, C-T. Ho, "Novel glycosides from noni (morinda citrifolia)," *J. Nat. Prod*, vol. 63, no. 8, pp. 1182-1183, 2000.

[5] B. Tan, N. Chiranthanut, S. Chansakaow, S. Sireeratawong, P. Khonsung, W. Nimlamool, M.N. Takuathung, and N. Lertprasertsuke, "Anti-inflammatory effects of pikad tri-phol-sa-mut-than remedy, consisting of dried fruits of aegle marmelos (L.) corrêa, coriandrum sativum L. and morinda citrifolia L," *Journal of Ethnopharmacology*, vol 298, p. 115639, 2022.

[6] B.F. Wahidah, F. Husain, "Etnobotani tumbuhan obat yang dimanfaatkan oleh masyarakat desa samata kecamatan somba opu kabupaten gowa sulawesi selatan," *Life Science*, vol. 7, no. 2, pp. 56-65, 2018.

[7] E. Dussossoy, P. Brat, E. Bony, F. Boudard, P. Poucheret, C. Mertz, J. Giaimis, and A. Michel, "Characterization, anti-oxidative and anti-inflammatory efects of costa rican noni juice (morinda citrifolia L.)," *Journal of Ethnopharmacology*, vol. 133, pp. 108–115, 2011.

[8] L. Lv, H. Chen, C.T. Ho, and S. Sang, "Chemical components of the roots of the noni (morinda citrifolia) and their cytotoxic effects," *Fitoterapia*, vol. 82, pp. 704-708, 2011.

[9] E.S. Almeida, M.A. Knapp, J.D.G. Rocha, D. Hotza, and D. Oliveira, "Multi-Stage block freeze concentration via gravitational method applied to increase of the nutritional content of morinda citrifolia L. tea," *Food Bioscience*, vol. 41, 102295, 2023.

[10] T.R. Marques, M.A. Braga, P.H.S. Cesar, N.P de Souza, R.M. Fraguas, D.A. Oliviera, M.V.C. Trento, S. Marcussi, "Evaluation of the brazilian functional fruit morinda citrifolia as phospholipases A2 and proteases modulator

morinda citrifolia: enzymatic modulation," *Phytomedicine Plus*, vol. 1, p. 100071, 2021.
[11] Y. Deng, Y.W. Chin, H. Chai, W.J. Keller, and A. D. Kinghorn, "Anthraquinones with quinone reductase-inducing activity and benzophenones from morinda citrifolia (noni) roots, *J. Nat. Prod*., vol. 70, pp. 2049–2052, 2007
[12] E.I. Taskın, K. Akgun-Dar, A. Kapucu, E. Osanc, H. Dogruman, H. Eraltan, and E. Ulukaya, "Apoptosis-Inducing effects of morinda citrifolia l. and doxorubicin on the ehrlich ascites tumor in balb-c mice," *Cell Biochem Funct*., vol. 27, pp. 542–546, 2009
[13] Ferlinahayati, R. Apriyaty and Eliza, "Fatty Acid and Alkenil Glycoside from the Fruits of mengkudu (morinda citrifolia linn)," *Indonesian Journal of Fundamental and Apllied Chemistry*, vol 1, no 2, pp. 52-54, 2016.
[14] Ferlinahayati, Y.M. Syah, L.D. Juliawaty, and E.H. Hakim, "Flavanones from the wood of morus nigra with cytotoxic activity," *Indo. J. Chem*., vol 13, no 3, pp. 205 – 208, 2013.
[15] D.L Pavia, G.M. Lampman, G.S. Kriz, and J.R. Vyvyan, "Introduction *to Spectroscopy*, 5th ed. Cengage Learning, 2015.
[16] B.S. Siddiqui, F.A. Sattar, F. Ahmad and S. Begum, "Isolation and structural elucidation of chemical constituents from the fruits of morinda citrifolia linn," *Arch Pharm Res*., vol. 30, no. 8, pp. 919-923, 2007.
[17] K.S. Vidya, U. Raghavendra, A. Muthusamy, S.B. Vidhu, J. Subramani, P.M. Gopinath, and K. Satyamoorthy, "Cytotoxic efficacy of noni (morinda citrifolia l.) fruits extract and scopoletin on a preponredant panel of human tumor cell lines," *Intl. J. Noni. Res*, vol 7, no 1, pp. 62-72, 2012.
[18] W. Thani, O. Vallisuta, P. Siripong, and N. Ruangsises, "Anti Proliferative and Antioxidative Activities of Thai Noni/Yor (Morinda citrifolia Linn.) Leaf Extract," *Southeast Asian J. Trop. Med. Public Health*, vol. 41, no. 2, pp 482-489, 2010.